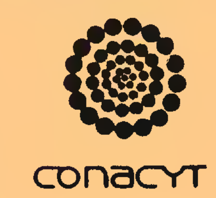
ISSN 0187-7151



ACTA BOTANICA MEXICANA

núm. 60 *Octubre 2002*

Instituto de Ecología A.C.



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Acta Botanica Mexicana (2002), 60: 1-5

DOS NUEVOS REGISTROS PARA LA FLORA DE MUSGOS DE COSTA RICA: *STEEREOBRYON SUBULIROSTRUM* Y *POGONATUM PENSILVANICUM* (POLYTRICHACEAE)

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RESUMEN

Como resultado de exploraciones durante el Primer Curso de "Briología Tropical" de la Red Latinoamericana de Botánica, se recolectaron dos especies de Polytrichaceae que son nuevas para la flora de musgos de Costa Rica y América Central: *Steereobryon subulirostrum* (Schimp. ex Besch.) G. L. Sm. y *Pogonatum pensilvanicum* (Hedw.) P. Beauv. El primer taxon además representa un género no conocido previamente de Centroamérica. En tal virtud, se proveen claves para la identificación de los géneros de Polytrichaceae y de las especies de *Pogonatum* de Costa Rica.

Palabras clave: briofitas, Costa Rica, florística, musgos, Polytrichaceae.

ABSTRACT

As a result of field trips during the first "Tropical Bryology" course of the Red Latinoamericana de Botánica, we collected two species of Polytrichaceae that are new to the moss flora of Costa Rica and Central America: *Steereobryon subulirostrum* (Schimp. ex Besch.) G. L. Sm. and *Pogonatum pensilvanicum* (Hedw.) P. Beauv. The former taxon represents also a genus previously not known from Central America. In this connection we provide two identification keys, one for the genera of Polytrichaceae, and another for species of *Pogonatum* in Costa Rica.

Key words: bryophytes, Costa Rica, floristics, mosses, Polytrichaceae.

INTRODUCCIÓN

Entre los países centroamericanos, posiblemente la flora de musgos de Costa Rica es la mejor conocida y la más diversa. Inicialmente Bowers (1974) reconoció 542 taxa. Los registros adicionales de Crosby (1974) y Morales (1982), así como los rearreglos taxonómicos y nomenclaturales modernos, permiten reajustar la lista a más de 620 especies de musgos (Dauphin y Morales, com pers.; Delgadillo et al. 1995).

Gracias al apoyo de la Red Latinoamericana de Botánica (RLB), un grupo de briólogos latinoamericanos participamos en una excursión a la estación biológica “Alberto M. Brenes”, en la región central de Costa Rica. Este viaje de campo se realizó como parte del primer curso de “Briología Tropical” de la RLB, efectuado en la Universidad de Costa Rica, San José. Entre las colecciones destacan las que aquí se comunican por representar dos adiciones a la flora conocida de los musgos del mencionado país.

RESULTADOS Y DISCUSIÓN

Steereobryon subulirostrum (Schimp. ex Besch.) G. L. Sm., Mem. New York Bot. Gard. 21: 56. 1971.

Espécimen y localidad: *De Luna 2576* (USJ, XAL), Alajuela, Estación Biológica “A. M. Brenes”, UCR. NE de San Ramón. Bosque premontano nuboso con Lauraceae, *Heliocarpus*, *Wercklea* y estrato arbustivo muy desarrollado. A la orilla del camino. Sobre suelo entre rocas bajas, sitio expuesto y húmedo. Altitud ca. 800 m. Febrero 16, 2001.

Este es el primer registro del género para Costa Rica y para Centroamérica. Todavía no se ha resuelto si se debe reconocer dos especies de *Steereobryon* o una sola. Por un lado Crosby et al. (1992) y Churchill et al. (2000) listan a: *S. elamellosum* (Herzog) Menzel y *S. subulirostrum*, ambas distribuidas en el Neotrópico. Sin embargo, Smith (1994) e Hyvönen et al. (1998) aluden al género como monotípico, reconociendo solamente a *S. subulirostrum.*

Si *Steereobryon* (Polytrichaceae) tiene dos especies, entonces *S. elamellosum* (Herzog) Menzel no se ha recolectado en ningún otro país (Churchill et al., 2000) desde que se describió originalmente de Bolivia. Delgadillo et al. (1995) la citan como *Atrichum elamellosum* (Herzog) Frye & Duck., aunque el taxon ya se había transferido a *Steereobryon* (cf. Crosby et al. 1992; Smith, 1994). Se puede hallar una descripción detallada e ilustraciones de *S. elamellosum* en Frye y Duckering (1947).

La segunda especie del género es *S. subulirostrum.* Se ha recolectado en Colombia, Ecuador, Venezuela (Churchil y Linares, 1995), Perú (Churchill et al., 2000), México (Smith, 1994), República Dominicana (Buck y Steere, 1983) y Puerto Rico (Sastré De Jesús y Buck, 1993). Además, Smith (1971) la cita de Jamaica, aunque Delgadillo et al. (1995) omiten este dato, por lo que su presencia en Jamaica debe ser confirmada. El registro de *S. subulirostrum* de Centroamérica se encontraba en una situación confusa similar. Smith (1971) no cita ningún país de esta parte del continente en la distribución del taxon. Después, Smith (1994) y Delgadillo et al. (1995) lo aceptaron como ya registrado de América Central, pero no especificaron el país. Ahora se ha recolectado en

Costa Rica y se confirma así su presencia en el área. Se pueden hallar descripciones detalladas e ilustraciones de *S. subulirostrum* en Frye (1948) y Smith (1971, 1994).

Con objeto de facilitar la identificación de los géneros de Polytrichaceae conocidos de Costa Rica, presentamos una clave, adaptada de la de Griffin y Morales (1983) y la propuesta por Churchill y Linares (1995) con algunas modificaciones. Esta clave incluye *Steereobryon* y los seis géneros previamente conocidos de Costa Rica (Delgadillo et al. 1995).

Clave para los géneros de Polytrichaceae de Costa Rica

1 Márgenes de las hojas bordeadas por células alargadas 2
1 Márgenes de las hojas con células cortas, semejantes a las del resto de la lámina 3
  2 Márgenes de las hojas con dientes simples; células del margen levemente pluripapilosas .... *Steereobryon*
  2 Márgenes con dientes dobles; células del margen lisas, sin papilas .... *Atrichum*
3 Márgenes de la hoja doblados longitudinalmente, cubriendo la superficie adaxial de la lámina; células terminales de las lamelas hendidas o piriformes .... *Polytrichum*
3 Márgenes planos o incurvados, superficie adaxial libre, células terminales de las lamelas de diversas formas, redondeadas, truncadas o piriformes 4
  4 Lamelas restringidas a la superficie dorsal de la costa .... *Oligotrichum*
  4 Lamelas sobre la costa y la superficie adaxial de la lámina 5
5 Hojas claramente diferenciadas en vaina y limbo, vaina obovada a oblongo-obovada; cápsula angular a prismática, peristoma con 64 dientes 6
5 Hojas levemente o no diferenciadas en vaina y limbo, vaina ovada; cápsula rolliza a cilíndrica, peristoma con 32 dientes .... *Pogonatum*
  6 Células terminales de las lamelas papilosas, vaina de la hoja semitranslúcida o verdosa; cápsulas con 4 ángulos; caliptra densamente pilosa .... *Polytrichastrum*
  6 Células terminales de las lamelas lisas, vaina de la hoja teñida de anaranjado; cápsulas con 2 ángulos; caliptra glabra o escasamente pilosa .... *Polytrichadelphus*

Pogonatum pensilvanicum (Hedw.) P. Beauv., Mém. Soc. Linn. Paris 1: 461. 1822.

Espécimen y localidad: *De Luna 2575*, (USJ, XAL), Alajuela, Estación Biológica "A. M. Brenes", UCR. NE de San Ramón. Bosque premontano nuboso con Lauraceae, *Heliocarpus*, *Wercklea* y estrato arbustivo muy desarrollado. A la orilla del camino. Sobre suelo, talud expuesto y húmedo. Altitud ca. 800 m. Febrero 16, 2001.

Este es el primer registro de *P. pensilvanicum* para Costa Rica y para Centroamérica. La especie se ha citado de Canadá, Estados Unidos, México, Perú, Bolivia, Brasil, Paraguay, Cuba (Hyvönen, 1989; Delgadillo et al., 1995), Venezuela (Ramírez y Crusco de Dall'Aglio, 1978) y Colombia (Churchill y Linares, 1995; Churchill et al., 2000). Se pueden encontrar descripciones e ilustraciones en publicaciones recientes como las de Crum y Anderson (1981, p. 1262 fig. 627 A-I), Hyvönen (1989), Smith (1994) y Churchill y Linares (1995). El habitat en Costa Rica, un talud a la orilla

del camino, corrobora la percepción de que *P. pensilvanicum* es común en ambientes ruderales y suelos expuestos.

Este taxon se agrega a otros del género ya conocidos en el área centroamericana. *Pogonatum* (Polytrichaceae) está formado por unas 52 especies en el mundo (Hyvönen, 1989), de las cuales, unas 10 son propias del Neotrópico. Para Costa Rica solamente se sabía de la existencia de cuatro: *P. campylocarpum* (Müll. Hal.) Mitt., *P. comosum* (Müll. Hal.) Mitt., *P. procerum* (Lindb.) Schimp. y *P. tortile* (Sw.) Brid. De Centroamérica se han registrado además otras dos: *P. perichaetiale* subsp. *oligodus* (Müll. Hal.) Hyvönen y *P. volvatum* (Müll. Hal.) Par. (Hyvönen 1989). La clave que se presenta a continuación para las cinco especies de *Pogonatum* conocidas de Costa Rica fue adaptada de Hyvönen (1989):

Clave para las especies de *Pogonatum* (Polytrichaceae) de Costa Rica

1 Hojas pequeñas o reducidas, lamelas vestigiales; protonema filamentoso persistente .... *P. pensilvanicum*

1 Hojas anchas y bien desarrolladas, lamelas usualmente bien desarrolladas; protonema efímero 2

2 Células apicales de las lamelas con paredes delgadas, isodiamétricas 3

2 Células apicales de las lamelas con paredes engrosadas, claramente más altas que anchas 4

3 Gametofitos robustos, células apicales de las lamelas geminadas (dobles) .... *P. procerum*

3 Gametofitos de tamaño mediano, células apicales de las lamelas mayormente sencillas .... *P. tortile*

4 Hojas fuertemente adpresas (en seco), vaina con hombros sobresalientes, margen de la base entero; lámina con constricción basal acentuada; células apicales de las lamelas con la pared externa engrosada .... *P. comosum*

4 Hojas libremente curvadas (en seco), vaina sin hombros, márgenes de la base aserrados; lámina con constricción basal débil o ausente; células apicales de las lamelas con la pared externa ligeramente engrosada .... *P. campylocarpum*

Las dos especies de Polytrichaceae tratadas en este trabajo son abundantes localmente en la estación biológica “A. M. Brenes”. Dado el ambiente y el tipo de vegetación donde se recolectaron, es de esperarse que ambas tengan una distribución más amplia, especialmente en las regiones central y occidental de Costa Rica. Los presentes registros llenan parcialmente el vacío de la distribución conocida de ambas especies entre Sudamérica y Norteamérica.

AGRADECIMIENTOS

Las colecciones se efectuaron con financiamiento de la Red Latinoamericana de Botánica para el curso “Briología Tropical”, realizado en San José, del 11 al 22 de febrero, 2001. Apreciamos mucho el apoyo recibido de M. I. Morales para visitar la estación

biológica “A. M. Brenes” y su ayuda en el envío de especímenes. Agradecemos los comentarios de Jaakko Hyvönen y Maria Isabel Morales y también las correcciones de dos revisores anónimos que mejoraron el manuscrito.

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Recibido en julio de 2001.
Aceptado en junio de 2002.

Acta Botanica Mexicana (2002), 60: 7-11

ELEOCHARIS OBTUSETRIGONA (CYPERACEAE) NEW TO NORTH AND CENTRAL AMERICA

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ABSTRACT

Eleocharis obtusetrigona (Lindl. & Nees) Steud., previously known only from South America, is recorded here for southeastern United States, Mexico and Central America. A description of the species is presented, as well as a key to separate *E. obtusetrigona* from other robust species of subgenus *Limnochloa* with which it has been confused.

Key words: Central America, Cyperaceae, distribution, *Eleocharis*, Mexico, new record, U.S.A.

RESUMEN

Eleocharis obtusetrigona (Lindl. & Nees) Steud., conocido previamente de Sudamérica, es registrado aquí para el sureste de los Estados Unidos, México y Centroamérica. Se presenta una descripción de la especie, así como una clave para especies robustas de *Eleocharis* subgénero *Limnochloa* con las cuales *E. obtusetrigona* ha sido confundida.

Palabras clave: Centroamérica, Cyperaceae, distribución, *Eleocharis*, México, nuevo registro, Estados Unidos de América.

During the preparation of the treatment of *Eleocharis* subgenus *Limnochloa* (Cyperaceae) for the Flora of North America project we discovered several collections of a species not previously recorded for the area. Some of those collections had been identified as *E. fistulosa* (Poiret) Link, an illegitimate name for *E. fistulosa* (Poiret) Schult. in Roem. & Schult., which is a synonym of *E. acutangula* (Roxb.) Schult. in Roem. & Schult.

Eleocharis acutangula is a common species in tropical America. Two of those collections were the bases on which *E. fistulosa* was cited from North America by Svenson (1957) and Correll & Correll (1975). However, these specimens differ from *E. acutangula* in several characters and belong instead to *E. obtusetrigona* (Lindl. & Nees) Steudel, which has previously been known only from South America. They are the first record of *E. obtusetrigona* for North America. Other collections of *E. obtusetrigona* which have been misdetermined as *E. fistulosa*, *E. cellulosa* Torr. and *E. quadrangulata* (Michx.) Roem. & Schult. are known from Mexico and Central America.

Eleocharis is a widely distributed genus of about 200 species. *Eleocharis obtusetrigona* belongs to *Eleocharis* subgenus *Limnochloa* (P. Beauv. ex T. Lestib.) Torr. (Ann. Lyceum Nat. Hist. New York 3: 296. 1836), which is equivalent to *E.* ser. *Mutatae* Svenson (Rhodora 31: 127. 1929). The main center of diversity of *E.* subgenus *Limnochloa* is tropical South America where 10 species are known (González Elizondo & Tena, 2000), with secondary centers in eastern North America and in Mexico.

Svenson (1939) placed *E. obtusetrigona* in *E. fistulosa* (*E. acutangula*). Clarke (1898) reduced it to *E. mutata* var. *obtusetrigona* (Steudel) Clarke, and Barros (1960) treated it as *E. fistulosa* var. *obtusetrigona* (Steudel) Barros. However, *E. obtusetrigona* can be separated by the cross-section shape of the culms, which are subterete or obscurely angled in *E. obtusetrigona* and sharply triangular in *E. acutangula* and *E. mutata*. Also, in general terms the glumes and achenes are larger in *E. obtusetrigona*, and the colour of the sheaths is proximally stramineous to pale rose, distally orange-reddish, salmon-coloured or purple in that species, and stramineous to brown or purple in *E. acutangula* and *E. mutata.* Plants of *Eleocharis obtusetrigona* have also been misidentified as *E. cellulosa* Torr. and *E. quadrangulata* (Michx.) Roem. & Schult. The following key distinguishes these species.

1 Culms terete or obscurely 3-5-angled; sheaths distally orange-reddish, salmon-coloured or purple; achene with 10-24 rows of cells each face 2

2 Achene with a stout, spongy thickening at the summit; tubercle 0.1-0.5 x 0.2-0.5 mm, confluent with or gradually merging from the summit of the achene; bristles smooth or finelly spinulous; floral scales broadly obovate to suborbicular .... *E. cellulosa*

2 Achene with a narrow, short neck at the summit or the neck obscure; tubercle 0.8-1.1 x 0.7-0.9 mm, not confluent with nor gradually merging from the summit of the achene; bristles usually coarsely spinulous; floral scales ovate to oblong .... *E. obtusetrigona*

1 Culms acutely 3 or 4-angled; sheaths distally stramineous, brown or purple; achene with 11-38 rows of cells each face 3

3 Culms acutely 4-angled; achene with 19-38 rows of cells each face to almost smooth .... *E. quadrangulata*

3 Culms acutely 3-angled; achene with 11-32 rows of cells each face 4

4 Floral scales ovate to oblong, coarsely nerved; achene with 11-15 rows of cells each face; tubercle proximally wider than the summit of the achene .... *E. acutangula*

4 Floral scales obovate to broadly suborbicular, finely nerved; achene with (16)24-32 rows of cells each face; tubercle confluent with or gradually merging from the summit of the achene .... *E. mutata*

Eleocharis obtusetrigona (Lindl. & Nees) Steud., Syn. pl. glumac. 2: 80. 1854. *Limnochloa obtusetrigona* Lindl. & Nees, in Mart. Fl. Bras. ii. 100. 1842; *Eleocharis mutata* (L.) Roem. & Schult. var. *obtusetrigona* (Lindl. & Nees) Clarke, Bull. Herb. Boiss. 6 (Append. 1): 20. 1898; *Eleocharis fistulosa* (Poiret) Schult. in Roem. & Schult. var. *obtusetrigona* (Lindl. & Nees) M. Barros, Sellowia 12: 262. 1960.- Type: Brasil, Bahia, *Salzmann* in *hb. Lindley* (CGE). *Eleocharis strobilacea* Pedersen. Darwiniana 12(2): 243. 1961.- Type: Argentina, Prov. Corrientes, *Pedersen 957* (C).

Perennial plants 40-110 cm. Rhizomes (often lacking in herbarium specimens) horizontal, creeping, 3-4 mm thick, soft to hard; longer internodes ca. 3-8 cm, reddish brown, smooth or slightly sulcate; tubers absent. Culms obscurely 3-5-angled to terete; 3.6-7.5 mm wide, or as narrow as 2 mm at the apex; soft; not septate, internally spongy, transverse septa incomplete. Distal leaf sheaths persistent, proximal leaf sheaths sometimes decaying, membranous, proximally stramineous to pale rose, distally orange-reddish, salmon-coloured or purple, sometimes open on the ventral side, distal sheath summit brownish or reddish to purple, often hyaline, not inflate or slightly inflate, oblique (angle as seen from the side ca. 45-75 deg. to the horizontal), acute to long acuminate, sometimes prolonged into a blade-like portion to 6 cm. Spikelets about 1.2-1.5 times wider than culm, cylindric to ovate, terete in cross section, obtuse to acute; 12-43 x 3.5-6 mm; proximal scale without a flower, amplexicaul, 3.6-7.5 mm, appearing to be a continuation of the culm. Floral scales ca. 30-125, ca. 25-36 per cm of rachilla, subappressed at the base, spiraled, deciduous, 4.3-5.8 x 2.5-3.3 mm, ovate to oblong; cartilaginous, often membranous toward margins; midnerve scarcely differentiated from the numerous, closely spaced and raised lateral nerves, midrib region nearly flat, greenish to pale brown or grayish, often with a submarginal reddish band or a subapical darker spot; flanks lighter, whitish to pale flesh-coloured, apices rounded to acute, 0.1-0.5 mm, colourless and hyaline, easily torn, margins broadly membranous-hyaline, colourless or pale brown. Perianth bristles ca. 6-7; stramineous with margins and spinules reddish to pale brown; stout, flattened; subequal, exceeding achene, 2.5-3.2 mm; coarsely retrorsely spinulose (rarely with delicate teeth), the spinules about 0.1 mm long. Anthers reddish brown, 1.5-1.9 x ca 0.2 mm, apex apiculate. Styles trifid (sometimes bifid?), puberulent. Tubercles stramineous to yellow-brown; lamelliform to high-pyramidal, sometimes spongy; 0.8-1.1 x 0.7-0.9 mm. Achenes stramineous often ripening to pale brown or greenish, shiny; obpyriform; biconvex; summit often constricted into a neck 0.5-0.6 mm wide, or the neck reduced and inconspicuous; 1.7-3 x (1-)1.2-1.5 mm; at 10-15 times magnification clearly sculptured, each face with 10-18 rows of transversely elongated cells, often isodiametric at achene base.

Eleocharis obtusetrigona is known from southeastern Texas, eastern Mexico, Central America and eastern South America. In Texas it forms large colonies in water in sites where water stands throughout the year at elevations of 0-20 m. In Mexico and Central America it grows up to 1200 m, in fresh permanent water in ditches, pools of standing water and border of roads. Flowering late winter to fall, fruiting spring to fall.

Although locally abundant and forming clonal masses, it is very local in North and Central America, but is more widely distributed in eastern Brazil and northern Argentina

and Paraguay (Barros, 1960; Pedersen, 1961). This kind of disjunct distribution suggests that *E. obtusetrigona* has been introduced from South America by several events (not necessarily recent) of long-distance dispersal. A more disjunct distribution between southeastern Texas and northern Argentina and Paraguay was reported by Guaglianone & Ueno (1990) for *Eleocharis cylindrica* Buckley.

Eleocharis obtusetrigona is known from North and Central America from the following collections:

U.S.A., Texas: Cameron County, Laguna Atascosa National Wildlife Refuge, ca. 0.9 mi S of entrance, near main road, in water, 23-04-1959, *A. Traverse 1060* (TEX); Cameron County, four miles east of Rio Hondo, 8-02-1934, *E. U. Clover 1752* (MICH, TEX); Brownsville, south of Armstrong, in Kennedy County near highway 96, large colonies in shallow water, 17-10-1938, *R. Runyon 1953* (TEX); Brownsville, 20 miles North of Brownsville, bordering the Paredes road, wet ground, ditches, etc., it forms large colonies, 16-06-1941, *R. Runyon 2765* (TEX); Brownsville, 20 miles North of Brownsville, on the Paredes road, in ditches where water stands through year, it forms large colonies in water, 6-04-1944, *R. Runyon 3680* (TEX).

MEXICO, Veracruz: Miradores, municipio de Dos Ríos, 14-10-1974, *F. Ventura 10639* (CIIDIR, ENCB-2); Rancho de la Secretaría de Salubridad y Asistencia (antes Rancho Tres Pasos), municipio de Emiliano Zapata, 19°32' N, 96°50' W, 15-04-1977, *R. V. Ortega 673* (IEB, MEXU).

HONDURAS: vicinity Agua Amarilla and Hoya Grande, Depto. Fco. Morazán, 27-10-1989, "tul", *A. Molina 34176* (MEXU).

COSTA RICA: Laguna Cóncavas, Prov. Cártago, Cantón Paraíso, 22-08-1983, *A. Lot* y *A. Novelo 1214 bis* con *R. Soto* (MEXU).

NICARAGUA: Estelí, along carretera Norte, 11 km north of Estelí, 13-08-1978, *P. C. Vincelli 858* (CIIDIR, MEXU, MO).

ACKNOWLEDGEMENTS

Thanks are given to the late Dr. T. M. Pedersen for sharing his expertise regarding the variation and distribution of *E. obtusetrigona* in South America; to Dr. A. A. Reznicek, M.C. Eleazar Carranza, Dr. P. M. Peterson, and two anonymous reviewers for comments; and to the curators of CIIDIR, ENCB, IEB, MEXU, MICH, MO, NY and TEX/LL herbariums for loans of specimens. M. González and S. González acknowledge to CGPI, COFAA and EDI of the Instituto Politécnico Nacional for support for research.

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Recibido en noviembre de 2001.
Aceptado en junio de 2002.

Acta Botanica Mexicana (2002), 60: 13-18

UNA NUEVA ESPECIE DE *RIBES* (GROSSULARIACEAE) DE MICHOACÁN, MÉXICO

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RESUMEN

Se propone como especie nueva para la ciencia a *Ribes grandisepalum*, un arbusto que habita en el bosque de *Abies* en el estado de Michoacán, México. Este taxon pertenece al subgénero *Coreosma* sección *Calobotrya* (Spach) Jancz. (Grossulariaceae) y se encuentra estrechamente relacionado con *Ribes pringlei* Rose. Se distingue de éste por presentar sépalos glanduloso-pubescentes, que alcanzan tamaños de 5.5-8.5 mm de largo, pedicelos de 7-17 mm de largo y pelos glandulares en el ovario y el fruto.

Palabras clave: Grossulariaceae, México, Michoacán, *Ribes*.

ABSTRACT

Ribes grandisepalum, a shrub that lives in the forest of *Abies* in the state of Michoacan, Mexico, is proposed as a new species to science. The new taxon belongs to subgenus *Coreosma* section *Calobotrya* (Spach) Jancz. (Grossulariaceae) and is related to *Ribes pringlei* Rose. It is distinguished by its glandular-pubescent sepals that reach sizes of 5.5-8.5 mm in length, pedicels 7-17 mm in length and the ovary and fruit with glandular hairs.

Key words: Grossulariaceae, Mexico, Michoacan, *Ribes*.

Al examinar ejemplares del género *Ribes* para la Flora de Veracruz (Durán-Espinosa, 2001), se detectaron especímenes determinados originalmente como *R. ciliatum,* cuyas características con respecto al tamaño de los sépalos y pedicelos, así como al indumento de los frutos, no correspondían a dicha especie (Cuadro 1). Por tal motivo, se realizó una revisión exhaustiva de los taxa de este género descritos para México y se llegó a la conclusión de que se trata de una especie nueva.

Ribes L. (Grossulariaceae) es un género cosmopolita con cerca de 150 taxa (Sinnott, 1985), incluye diversas especies cultivadas por sus frutos comestibles, conocidos como grosellas. La mayoría se encuentra en el hemisferio norte, aunque para la región de los Andes se han registrado 35. La delimitación de *Ribes* es controversial, debido a que en este género se reconocen dos grupos: el que incluye plantas espinescentes con unos cuantos racimos de flores escasas (uva-espinas) y el constituido por taxa carentes de espinas con racimos de numerosas flores (grosellas). Tales conjuntos son reconocidos indistintamente como dos géneros: *Grossularia* y *Ribes* (Sponberg, 1972); o bien, dentro de *Ribes* como subgéneros (Coville y Briton 1908; Berger, 1924; Poyarkova 1939; Sinnott 1985). Con estudios recientes sobre filogenias moleculares se han detectado también dos grupos (Messinger et al., 1999); sin embargo, no existe consenso en la circunscripción de los mismos.

Ribes grandisepalum C. Durán-Espinosa & S. Avendaño, sp. nov. Fig. 1.

Frutex ad 4 m altus; folia 2-6.5 cm longa et lata, palmata, 3-5 lobata, alternatim disposita; inflorescentiae 3.5-7 cm longae, racemosae, glanduloso-pubescentes, pedicelli 7-17 mm longi, basaliter bracteati, bracteae 7-26.5 mm longae, lanceolatae vel foliaceae; flores albidi vel subrosei; hypanthium 6.5-10 mm longum, 4.5-8.5 mm latum, tubulosum; sepala 5.5-8.5 mm longa, extus glanduloso-pubescentia, petala 3.8 mm longa, glabra; stamina linearia, filamenta 4.3-5 mm longa, antherae oblongae, basifixae; ovarium 4.5-6 mm longum, inferum, pyriforme, glandulosum; stylus 10.5 mm longus, furcatus ad apicem; fructus 8.3-9 mm longi, 5.2-5.5 mm lati, baccati, rubelli vel purpurati, glandulosi; semina ellipsoidea, numerosa.

Arbusto de 2.5-4 m de alto, sin espinas, con la corteza fisurada, las ramas cuando adultas escasamente glanduloso-pubescentes o glabras. Hojas alternas, pecioladas, palmadamente lobadas, de 2-6.5 cm de largo, 2-6.5 cm de ancho, con 3-5 lóbulos agudos, cuando presentan 5 lóbulos, los 2 basales más pequeños que los 2 intermedios y el apical, el lóbulo apical tan grande como los lóbulos intermedios o frecuentemente duplicando su tamaño, margen irregularmente dentado, glanduloso-pubescente, ápice agudo, base subcordada, senos abiertos, superficie adaxial con pelos glandulares esparcidos, la abaxial glanduloso-pubescente sobre todo a lo largo de las venas, la venación actinódroma marginal, sumergida en el haz, sobresaliente en el envés, pecíolo de 1.5-4 cm de largo, 0.4-0.8 mm de ancho, glanduloso-pubescente, base dilatada, envainante, con pelos glandulares hasta de 1.5 mm de largo. Inflorescencia racemosa, de ca. 12 flores, de 3.5-7 cm de largo, glanduloso-pubescente, pedúnculo de 3-4.3 cm de largo, 0.9-1.1 mm de ancho. Flores blanquecinas a rosadas, pedicelos de 7-17 mm de largo, 0.4-0.5 mm de ancho, glanduloso-pubescentes, brácteas lanceoladas a foliáceas, ápice acuminado, sésiles, de 7-26.5 mm de largo, 2-7.5 mm de ancho, glanduloso-pubescentes, bracteolas lanceoladas, ápice acuminado, sésiles, de 2.5-6 mm de largo, 0.3-0.8 mm de ancho, glanduloso-pubescentes; hipantio tubular, de 6.5-10 mm de largo, 4.5-8.5 mm de ancho, glanduloso-pubescente; sépalos 5, oblongos, de 5.5-8.5 mm de largo, 2.2-2.8 mm de ancho, dorsalmente glanduloso-pubescentes, ventralmente glabros; pétalos 5, orbiculares, de 3.8 mm de largo, 3.5 mm de ancho, glabros, alternando con los sépalos; estambres 5, lineares, incluidos, filamentos de 4.3-5 mm de largo, 0.2-

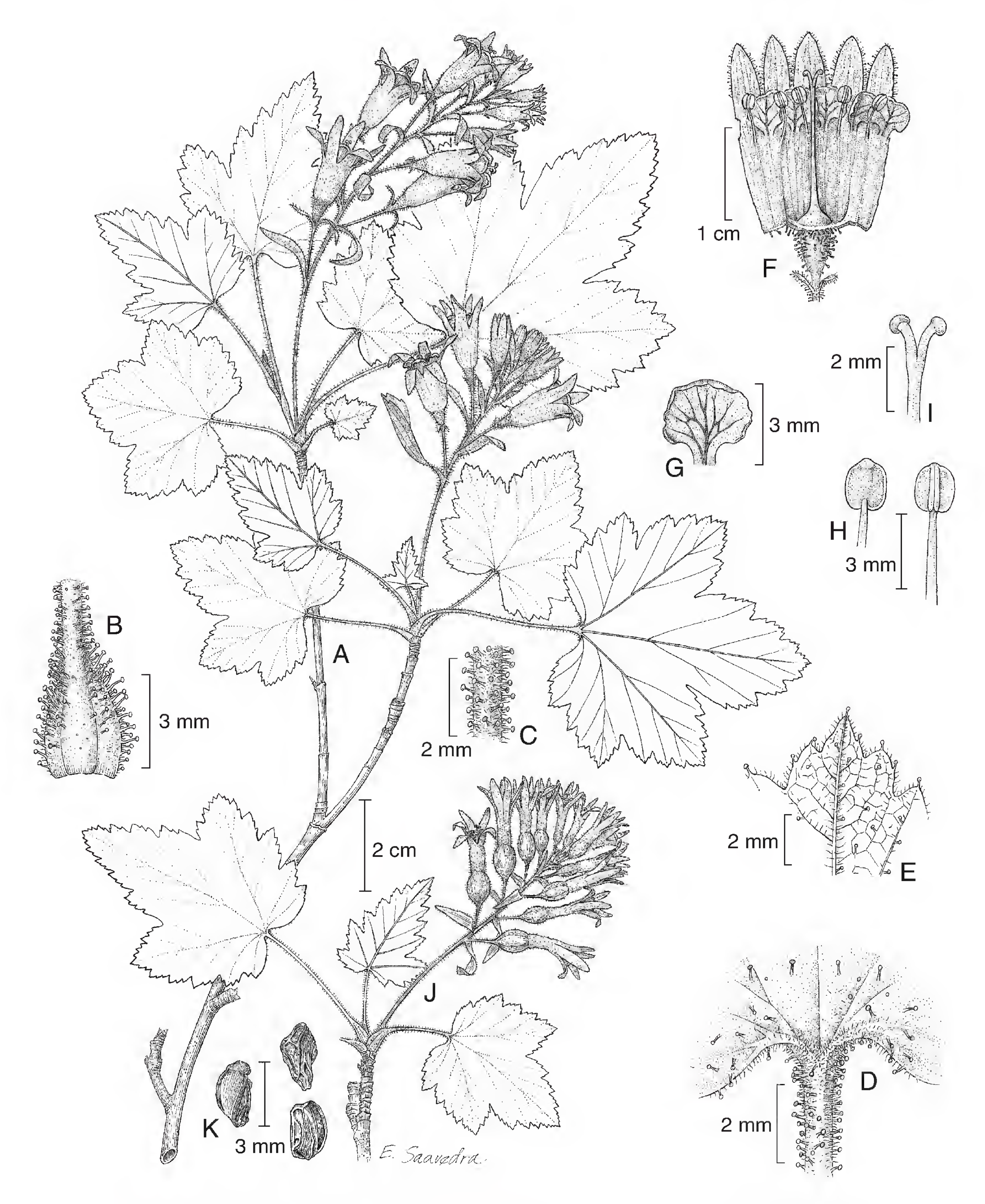


Fig. 1. *Ribes grandisepalum*. A. rama con inflorescencia; B. base del pecíolo de la hoja; C. fragmento de pecíolo; D. base de la hoja; E. superficie adaxial de la hoja; F. flor abierta; G. pétalo; H. estambres; I. estilo; J. rama con infrutescencia. Ilustración de E. Saavedra basada en el ejemplar de *Rzedowski 46282*.

0.3 mm de ancho, insertos internamente a la superficie de la copa floral por arriba de la mitad, alternando con los pétalos, anteras oblongas, de 1.3-1.6 mm de largo, 0.7-0.8 mm de ancho, basifijas, la dehiscencia longitudinal; ovario ínfero, piriforme, de 4.5-6 mm de largo, 3 mm de ancho, glandular, estilo de 10.5 mm de largo, 0.4-0.7 mm de ancho, bifurcado apicalmente, estigmas truncados. Fruto una baya, de color rojo a guinda, ovoide, de 8.3-9 mm de largo, 5.2-5.5 mm de ancho, glandular, con vestigios del cáliz; semillas negras, numerosas, de 2.2-3 mm de largo, 1-1.5 mm de ancho, elipsoides, regularmente comprimidas.

TIPO: México, Michoacán, mpio. Zinapécuaro, 1 km al NW de Laguna Larga, sobre el camino a Yerba Buena, *Rzedowski 46282* (Holotipo: XAL; Isotipos: IEB, MEXU).

Material adicional examinado. Mpio. Pátzcuaro, parte alta del Cerro del Burro, cerca de Cuanajo, *H. Díaz B. 1014* (MEXU); mpio. Huiramba, parte alta del cerro La Taza, *H. Díaz B. y S. Zamudio R. 2623* (IEB); mpio. Zinapécuaro, alrededores de Laguna Larga, Los Azufres, *H. Díaz B. 4681* (IEB, XAL); mpio. Santa Clara del Cobre, Cerro del Burro, *J. M. Escobedo 868, 1399* (IEB, MEXU, XAL); mpio. Santa Clara del Cobre, Cerro del Burro, *E. Pérez C. 48 y 270* (IEB); mpio. Queréndaro, Cañada del Real, cerca de San José de la Cumbre, *J. S. Martínez 1310* (IEB, XAL); mpio. Ciudad Hidalgo, paraje El Pino, cerca de Huajúmbaro, *J. S. Martínez 1450* (ENCB, IEB, XAL); mpio. Zinapécuaro, ladera SW del cerro San Andrés, *S. Zamudio R. 5519* (IEB).

El epíteto específico de este nuevo taxon se deriva del tamaño de los sépalos, carácter que lo distingue del resto de las especies descritas con anterioridad para México (Janczewski, 1907; Coville y Britton, 1908). Se le conoce solamente del estado de Michoacán, donde habita en el bosque de *Abies* en cañadas pronunciadas, en altitudes que van de los 2500 a los 3200 m s.n.m. Se le encuentra en floración entre los meses de marzo a mayo.

La especie más cercanamente relacionada corresponde a *R. pringlei* Rose, que se distribuye en el Estado de México y en el Distrito Federal. A este respecto cabe comentar que en el estudio más reciente de las especies de *Ribes* del Valle de México realizado por Calderón de Rzedowski (2001), se considera a dicha especie como sinónimo de *R. ciliatum*.

Aunque algunos caracteres de *R. grandisepalum* y *R. pringlei* son variables, como el tamaño del hipantio, de los sépalos y de los pedicelos de las flores, y en ocasiones pueden solaparse los intervalos entre una especie y otra, dentro del conjunto se manifiestan como taxa separados. Por otra parte, *R. pringlei* se diferencia de *R. ciliatum* porque presenta frutos glandulares, así como otros rasgos que pueden apreciarse en el Cuadro 1 (Coville y Britton, 1908; Janczewski, 1907).

AGRADECIMIENTOS

Agradecemos al Dr. Francisco Lorea Hernández por la revisión crítica del manuscrito y la traducción de la diagnosis al latín y al Sr. Edmundo Saavedra por la estupenda ilustración del ejemplar.

Cuadro 1. Características diferenciales comparativas entre *Ribes grandisepalum* y especies afines.

Caracteres	*Ribes grandisepalum* C. Durán-Espinosa & S. Avendaño	*Ribes pringlei* Rose	*Ribes ciliatum* Humb. & Bonpl. ex Roem. & Schult.	*Ribes affine* Kunth	*Ribes orizabae Rose*
Haz de la hoja	con pelos glandulares esparcidos	con pelos glandulares esparcidos	glanduloso a glabrado	escasamente pubescente a glabrado	glabro
Envés de la hoja	glanduloso-pubescente sobre las venas	glanduloso-pubescente sobre las venas	densamente glanduloso-pubescente	pubescente sobre las venas	glanduloso-pubescente sobre las venas
Pecíolos	glanduloso-pubescentes	glanduloso-pubescentes	densamente glanduloso-pubescentes	pubescentes	glanduloso-pubescentes
Hipantio largo (mm) ancho (mm)	 6.5-10 4.5-8.5	 6-8 3.5-4	 4-5	 2-3.5	 4
Sépalos largo (mm) pubescencia	 5.5-8.5 glanduloso-pubescentes	 4-6 glanduloso-pubescentes	 4.5 pubescentes	 2.5-4 pubescentes	 3.8 pubescentes
Color de las flores	blanquecino a rosado	verde pálido a púrpura	blanco-verdoso, rosado o crema	blanco a rosado	
Largo del pedicelo (mm)	7-17	3-12	4-7	3-8	5-8
Brácteas largo (mm) ancho (mm)	 7-26.5 2-7.5	 5.5-9.5 1.8-3	 4-9.2 0.7-3.3	 4.5-15 1-8	 6-7.5 1.5-1.9
Ovario	glandular	glandular	glabro	glabro	glabro
Fruto indumento	 glandular	 glandular	 glabro	 glabro	 glabro

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Recibido en agosto de 2001.
Aceptado en agosto de 2002.

Acta Botanica Mexicana (2002), 60: 19-24

VALIDATION OF *MICROEPIDENDRUM* (ORCHIDACEAE: LAELIINAE)

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ABSTRACT

The generic name *Microepidendrum* Brieger ex W. E. Higgins is validated, an epitype of *Epidendrum subulatifolium* A. Rich. et Gal. is selected, and a new combination *Microepidendrum subulatifolium* (A. Rich. et Gal.) W. E. Higgins is proposed. Other species included in *Microepidendrum* by Brieger are excluded from the group. A phylogeny of the subtribe Laeliinae is presented showing the genus *Microepidendrum* Brieger ex W. E. Higgins to be sister to all other genera in Laeliinae and not closely related to *Epidendrum* or *Encyclia*.

Key words: *Encyclia*, *Epidendrum*, Laeliinae, *Leptophyllum*, Mexico, *Microepidendrum*, *Oestlundia*, Orchidaceae, *Scaphyglottis*.

RESUMEN

Se valida el nombre genérico *Microepidendrum* Brieger ex W. E. Higgins, se selecciona un epitipo de *Epidendrum subulatifolium* A. Rich. et Gal. y se propone la nueva combinación *Microepidendrum subulatifolium* (A. Rich. et Gal.) W. E. Higgins. Otras especies adscritas por Brieger a *Microepidendrum* quedan excluidas del grupo. Se presenta una filogenia de la subtribu Laeliinae, en la cual se muestra que *Microepidendrum* Brieger ex W. E. Higgins es hermano de todos los demás géneros en Laeliinae y no está estrechamente relacionado ni con *Epidendrum* ni con *Encyclia*.

Palabras clave: *Encyclia*, *Epidendrum*, Laeliinae, *Leptophyllum*, México, *Microepidendrum*, *Oestlundia,* Orchidaceae, *Scaphyglottis*.

INTRODUCTION

Microepidendrum subulatifolium is an orchid that appears to be a small reed-stem *Epidendrum* with terete leaves (Fig. 1), but with a flower unlike *Epidendrum* or *Encyclia*. It is distributed in the dry oak forests of southern Mexico. This diminutive species, published as *Epidendrum subulatifolium* A. Rich. & Gal., and transferred to *Encyclia* by Dressler (1961), has no close allies in subtribe Laeliinae. A cladistic analysis based on holomorphology clearly shows that this species is neither an *Epidendrum* nor an *Encyclia* (Higgins, 2000). Holomorphology is the total collection of characters or the complete description of an organism including morphological, anatomical, chemical, and molecular

characteristics. A recent three-gene DNA phylogenetic study (Fig. 2) confirms these findings (Higgins, van den Berg and Whitten, in press). The only other name ever proposed for this species is *Microepidendrum subulatifolium* (A. Rich & Gal.) Brieger. However, when Brieger published the genus *Microepidendrum* (Brieger, 1977) he failed to provide a Latin diagnosis and to designate a type species, thus the name is invalid (Greuter et al., 2000). The generic name must be validated before the combination can be used.



Fig. 1. Drawing of *Microepidendrum subulatifolium* plant by Stig Dalström.

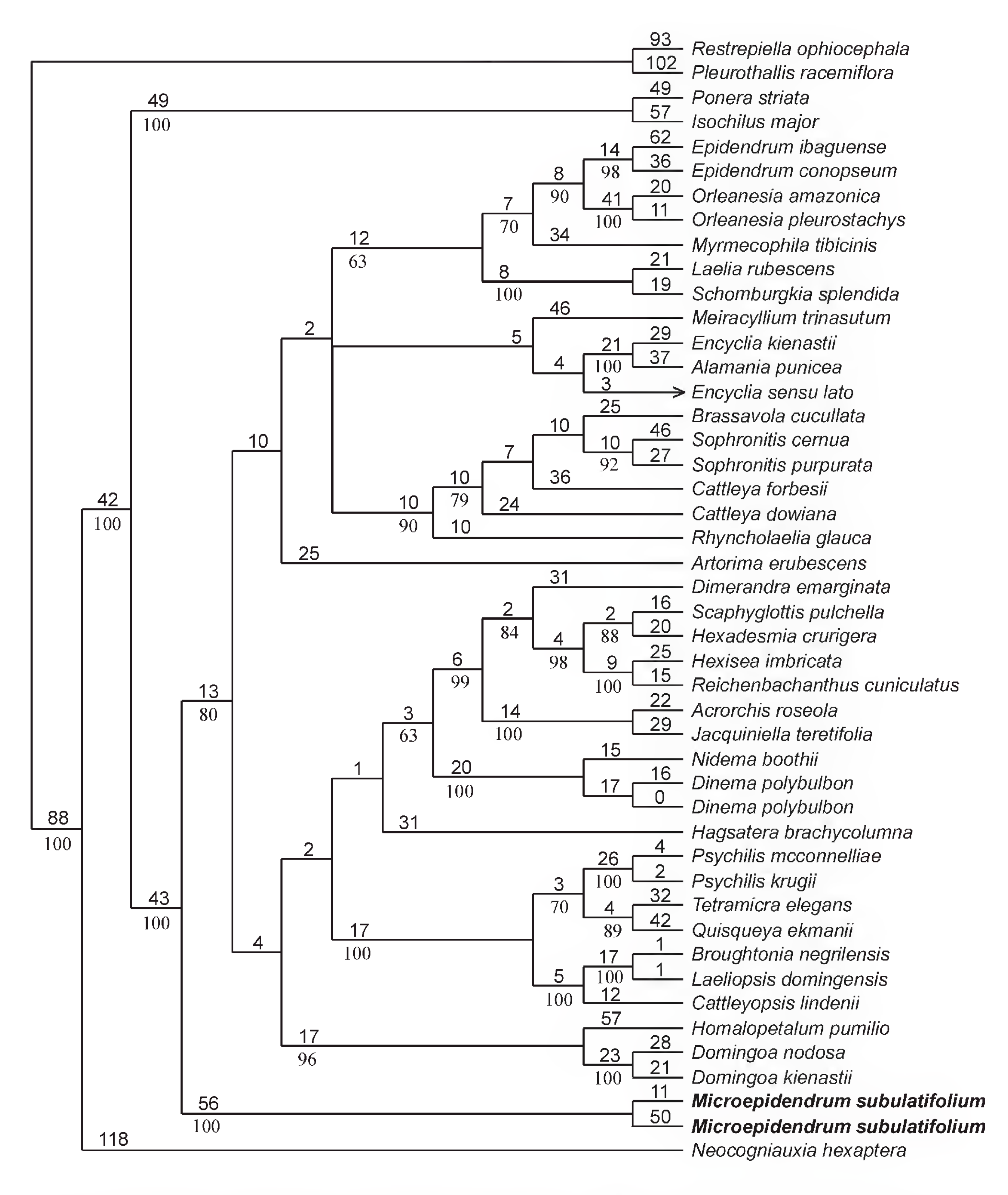


Fig. 2. Phylogeny of Laeliinae. The phylogenetic position of *Microepidendrum* is shown in a selected tree of a DNA Laeliinae topology. ACCTRAN character optimization was used to produce the Fitch branch lengths shown above the line with bootstrap indices shown below.

TAXONOMY

Microepidendrum Brieger ex W. E. Higgins, gen. nov.

Planta caespitosa, caulibus gracilibus; novis surculis et apicibus radicum ruberis; radicibus verrucosis incrassatis; foliis teretibus subulatis acutis; scapo terminali; labello suborbiculari unilobato adnato a columna.

Type species: *Epidendrum subulatifolium* A. Rich. & Gal.

Microepidendrum subulatifolium (A. Rich. & Gal.) W. E. Higgins comb. nov. Basionym: *Epidendrum subulatifolium* A. Rich. & Gal., Ann. Sci. Nat. III 3: 21 1845. Synonym: *Encyclia subulatifolia* (A. Rich. & Gal.) Dressler, Brittonia 13(3): 265. 1961.

Since the original *Epidendrum subulatifolium* type specimen, *Galeotti 5073* (W), is only a scrap of remaining vegetative material, an epitype is proposed here.

Epitype: *Hunt s. n.* Mexico: Jalisco, 3 km south of San José de Gracia (Michoacán) ca. 1700 m. (SEL 086578/OIC 13589).

Distribution: Mexico (Guerrero, Jalisco, Michoacan, Oaxaca).

Plant caespitose with very slender stems, 1-8 cm long, new growth red; roots thick, verrucose, root tip red; leaves 2-3 per stem, subcylindric, acute, strict, 2.5-12 cm long, 1.5-3 mm diameter; inflorescence racemose or paniculate, rachis flexuous, few-flowered, up to 25 cm long; sepals and petals yellow-brown, lip white, callus yellow, anther burgundy; sepals elliptic-lanceolate, complicate-acute, recurved, 6.5-8.5 mm long, 2-2.5 mm wide; petals oblanceolate-linear, acute or subacute, 5.5-8 mm long, 0.7-1 mm wide; lip adnate to 3/5 of column, simple, suborbicular, retuse, 9-9.5 mm long, 4-5.5 mm wide, strongly plicate-undulate, margins erose, reflexed; callus an ovate flattened disk with 3 papillose keels; column small, clavate, 4-4.5 mm long, three apical teeth subequal, midtooth obtuse surpassed by anther; capsule ellipsoid, 12 mm long, 5 mm wide. The flower of *Microepidendrum subulatifolium* is illustrated in Fig. 3.

The DNA phylogeny based on the *nr*ITS, *matK*, and *trnL-F* regions, presented in Fig. 2, shows *Microepidendrum* to be sister to the remainder of Laeliinae included in this analysis (Higgins, van den Berg and Whitten, in press). Two different specimens of the taxon that appear twice in the phylogenetic tree were used in this study in order to confirm their position in the subtribe. The numbers shown above lines are branch lengths, the number of synapomorphic DNA base changes, that support each clade and the bootstrap support indices are shown below. *Encyclia* sect. *Leptophyllum* Dressler & G.E. Pollard is polyphyletic. The other members of this section included in the *Encyclia* sensu lato clade of Fig. 2 have been moved to *Oestlundia* W.E. Higgins (Higgins, 2001).

Taxonomic note: The other species placed in *Microepidendrum* by Brieger are not closely related to *M. subulatifolium*. Their present position is as follows:

M. subliberum (C. Schweinf.) Brieger *nomen illegitimum* = *Scaphyglottis sublibera* (C. Schweinf.) Dressler.
M. serrulatum (Sw.) Brieger *nomen* = *Epidendrum serrulatum* Sw.
M. pallens (Rchb. f.) Brieger *nomen* = *Epidendrum pallens* Rchb. f.
M. miserrimum (Rchb. f.) Brieger *nomen* = *Epidendrum miserrimum* Rchb. f.
M. selaginella (Schltr.) Brieger *nomen* = *Epidendrum selaginella* Schltr.

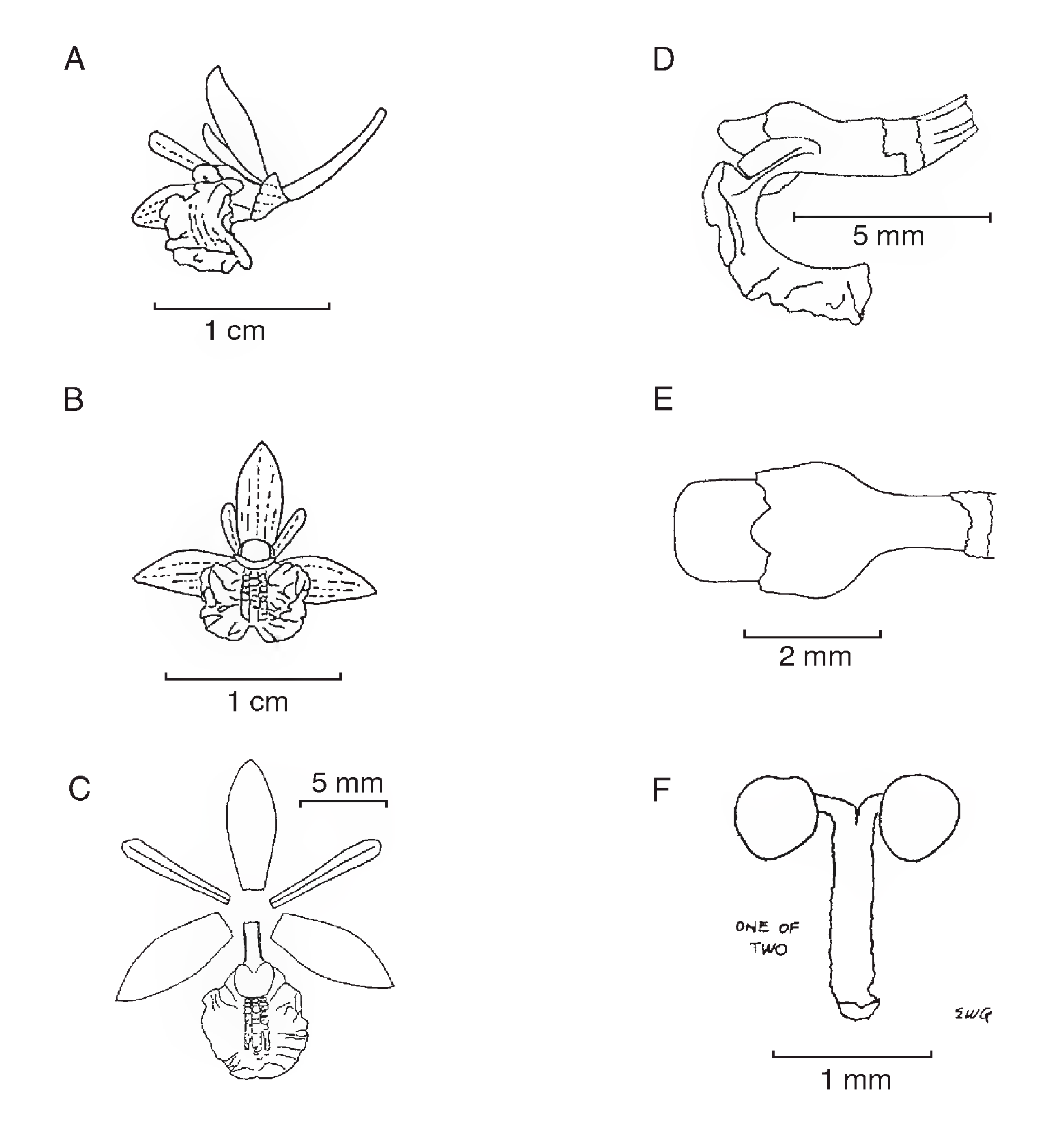


Fig. 3. Drawing of *Microepidendrum subulatifolium* flower by Ed W. Greenwood. A. quarter view of flower; B. front view of intact flower; C. dissected flower; D. side view of lip and column; E. top view of anther and column; F: two of four pollinia.

ACKNOWLEDGEMENTS

Thanks are given to Ed W. Greenwood who provided the flower illustration and John T. Atwood and to John Beckner for their comments on the manuscript.

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Recibido en mayo de 2001.
Aceptado en septiembre de 2002.

Acta Botanica Mexicana (2002), 60: 25-35

VIRIDANTHA, UN GÉNERO NUEVO DE BROMELIACEAE (TILLANDSIOIDEAE) ENDÉMICO DE MÉXICO

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RESUMEN

Se propone como nuevo para la ciencia el género *Viridantha* Espejo (Bromeliaceae), constituido por dos secciones y seis especies anteriormente adscritas a *Tillandsia*, endémicas de México; el género se caracteriza por presentar estambres todos iguales en longitud, filamentos aplanados, anteras de 2 a 4 mm de largo, subbasifijas, pétalos ligulados, libres, de color verde oscuro, flores proterandras, dísticas, escamas largamente asimétricas, densa a muy densamente dispuestas y estigmas del tipo simple-erecto (tipo I, sensu Brown & Gilmartin).

Palabras clave: Bromeliaceae, México, *Tillandsia plumosa*, *Viridantha*.

ABSTRACT

The genus *Viridantha* Espejo (Bromeliaceae) with two sections and six species, all endemic to Mexico and previously treated as *Tillandsia*, is proposed as new. *Viridantha* is characterized by the stamens equal in length, flat filaments and subbasifixed anthers, 2 - 4 mm long; ligulate, free, dark green petals; distichous, protandrous flowers; long asymmetric, densely to very densely disposed scales, and simple-erect stigmas (type I, sensu Brown & Gilmartin).

Key words: Bromeliaceae, Mexico, *Tillandsia plumosa*, *Viridantha*.

INTRODUCCIÓN

Las Tillandsioideae (Bromeliaceae) presentan como únicos caracteres en común el ovario súpero, o casi así y sus frutos capsulares, dehiscentes, conteniendo semillas plumoso-apendiculadas (Baker, 1889; Mez, 1896; Smith & Downs, 1977; Till, 2000a, b). Por lo demás, constituyen un grupo heterogéneo que incluye especies muy diferentes entre sí, tanto en los caracteres vegetativos como en los florales. Lo anterior es especialmente evidente en el complejo *Tillandsia*-*Vriesea*, en donde encontramos plantas que miden desde unos cuantos centímetros de alto, como *T. ionantha* Planch. hasta

verdaderos gigantes como *T. grandis* Schltdl., o plantas densamente escamosas y cinéreas como *T. plumosa* Baker hasta especies prácticamente glabras como *T. leiboldiana* Schltdl. o *V. heliconioides* (Kunth) Hook. ex Walp. En cuanto a la morfología floral, podemos encontrar diferencias en el tamaño y el color de las flores, el tamaño y la posición de los filamentos y las anteras, la disposición de los sépalos y los pétalos, la orientación de las flores en la antesis, etc., caracteres que indudablemente están relacionados con el proceso de polinización y la biología reproductiva de las especies y que por lo tanto influyen en su evolución y en sus relaciones filogenéticas.

La clasificación infragenérica en *Tillandsia* ha sido objeto de estudio por diversos especialistas en la familia, sin embargo no se ha logrado todavía un arreglo satisfactorio. Los subgéneros propuestos por autores como Baker (1889) y Mez (1896) (*Allardtia*, *Tillandsia*, *Strepsia*, *Diaphoranthema*, *Platystachys*, *Anoplophytum*, *Pseudocatopsis*, etc.) se basan en caracteres únicos como son la presencia o ausencia de escamas en los pétalos, las anteras erectas o versátiles o la condición exserto-inclusa de los estambres y/o estilo, o bien en caracteres tan poco claros como la variación foliar expresada por Baker (1889) como "differs ... only in leaf". Smith y Downs (1977) retomaron en su trabajo las propuestas de Baker y Mez y en algunos casos situaron especies fuera de lugar debido a que nunca vieron plantas vivas y por lo tanto no pudieron examinar las flores, lo cual aumentó el caos en las delimitaciones genéricas y/o subgenéricas.

La necesidad de revisar la circunscripción de los géneros y subgéneros dentro de las Tillandsioideae, utilizando para ello tanto caracteres florales como vegetativos, ha sido expresada por algunos autores (Gardner, 1982, 1986; Smith & Pittendrigh, 1953) y otros más, haciendo uso de los mismos, han llegado a segregar grupos definiendo géneros nuevos o restableciendo nombres genéricos anteriormente propuestos, como *Alcantarea*, *Werahuia* (Grant, 1995) y *Racinaea* (Spencer & Smith,1993).

Gardner (1982, 1986) revisó las especies mexicanas de *Tillandsia* subgénero *Tillandsia* (sensu Smith & Downs, 1977, pero incluyendo algunas del subgénero *Allardtia*), utilizando caracteres florales tomados de plantas vivas (79 especies de las 123 consideradas) y caracteres de los especímenes herborizados. Como resultado de su trabajo, propuso una división preliminar del subgénero en cinco grupos, sin dar nombre formal a ninguno de ellos.

Es claro que la clasificación genérica hasta ahora usada para el complejo *Tillandsia-Vriesea* deja mucho que desear y que, por otra parte, existen grupos que pueden ser delimitados por un conjunto de caracteres coherentes y consistentes, tanto florales como vegetativos, que reflejen mejor las relaciones filogenéticas y permitan definir un arreglo taxonómico más natural.

Uno de los grupos que Gardner (1982, 1986) reconoció en su trabajo es el de *Tillandsia plumosa* Baker y, aunque ella misma señaló que no corresponde estrictamente a *Tillandsia* subgénero *Tillandsia*, lo incluyó en su estudio, debido a que Smith y Downs (1977) habían considerado a dos de las seis especies del grupo: *T. ehrenbergii* Klotzsch ex Beer (= *T. tortilis* Klotzsch ex Baker) y *T. lepidosepala* L. B. Sm. dentro de dicho subgénero.

Los otros cuatro taxa del complejo fueron ubicados por los autores ya mencionados (Smith & Downs, 1977) en *Tillandsia* subgénero *Allardtia* y constituyen un ejemplo más de lo inadecuado de la clasificación subgenérica en *Tillandsia* porque, aunque todas ellas presentan estambres inclusos, en el resto de sus características

difieren notablemente de las propuestas como diagnósticas para el subgénero (Baker, 1889; Mez, 1896).

Tillandsia subgénero *Tillandsia*, tipificado por *Tillandsia polystachya* (L.) L. *(Renealmia polystachya* L.), se caracteriza por sus flores generalmente erectas, protóginas, con los pétalos espatulados, violáceos o verde-amarillentos, muy rara vez amarillos, rojos o blancos, de 4 a 6.8 cm de largo, constreñidos hacia el ápice alrededor de los estambres formando una garganta, por sus estambres exsertos, dispuestos en dos series de distinto tamaño, con los filamentos aplanados y ensanchados en el ápice y sus anteras versátiles. Además, las plantas del grupo presentan generalmente rosetas infundifbuliformes de tipo tanque y son por lo común glabras a glabrescentes (cf. Gardner, 1982, 1986).

En tanto que los caracteres que delimitan al grupo de *T. plumosa* son los siguientes: flores proterandras, dísticas, descendentes, con los pétalos ligulados, de color verde oscuro, de 2 a 3 cm de largo, no constreñidos para formar una garganta, estambres inclusos, todos iguales en longitud, con los filamentos aplanados, las anteras subbasifijas y el estigma del tipo simple-erecto (tipo I, sensu Brown & Gilmartin, 1984). Las rosetas en este conjunto son de tipo compacto, no forman tanque y tienden a la caulescencia, además de presentar escamas largamente asimétricas, densa a muy densamente dispuestas.

El grupo de *T. plumosa* se distingue así muy claramente de las demás especies de *Tillandsia* y presenta con el resto del complejo *Tillandsia-Vriesea* mayores diferencias que las existentes entre géneros como *Vriesea* y *Tillandsia*, *Racinaea* y *Tillandsia* o aun entre subfamilias como Tillandsioideae y Pitcairnioideae.

Cabe indicar aquí que algunas especies sudamericanas entre las que pueden citarse *T. tectorum* E. Morren, *T. heteromorpha* Mez, *T. vernicosa* Baker, *T. didisticha* (E. Morren) Baker y *T. comarapensis* H. Luther, comparten con el grupo de *T. plumosa* algunas características, como los estambres inclusos y del mismo largo, la forma y el tamaño de los pétalos, el denso indumento y la tendencia a la caulescencia, aunque difieren del mismo por presentar flores erectas, pétalos blancos o violáceos con el ápice blanco y estambres plicados.

Con base en lo anteriormente expuesto, se propone como nuevo el siguiente género:

Viridantha Espejo, genus novum

Herbae acaules vel caulescentes, epiphyticae vel rupicolae. Folia linearia. Squamae longialatae, asymmetricae. Inflorescentia pedunculata vel nidularis, simplex vel composita. Spicae applanatae. Flores distichi, proterandri, actinomorphi. Petala ligulata, libera, nuda, atroviridia. Stamina aequilonga, filamentis applanatis, antherae 2-4 mm longae, subbasifixae vel erectae. Stamina et pistillum in corolla inclusa. Stigma classi I teste Brown et Gilmartin. Ovarium superum. Capsula oblonga, acuminata, septicida. Semina basi appendiculata.

Hierbas acaules o caulescentes, epífitas o rupícolas, solitarias a rara vez cespitosas, xerófilas, ageotrópicas, de menos de 35 cm de alto. Hojas lineares. Escamas largamente aladas, asimétricas. Inflorescencia pedunculada a nidular, simple o compues-

ta. Espigas aplanadas. Flores dísticas, proterandras, horizontales a péndulas, actinomorfas, tubulares. Pétalos ligulados, libres, sin escamas, de color verde oscuro. Estambres todos del mismo largo, con los filamentos aplanados, anteras de 2 a 4 mm de largo, subbasifijas a erectas, amarillas o raramente negras. Estambres y pistilo más cortos que los pétalos, incluidos en la corola. Estigma del tipo simple-erecto (Tipo I, Brown & Gilmartin, 1984). Ovario súpero. Cápsula oblonga, acuminada, septicida, de color verde oscuro. Semillas con un apéndice plumoso en la base.

TYPUS: *Viridantha plumosa* (Baker) Espejo

El género es endémico del centro de México, donde se distribuye en los estados de Aguascalientes, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí y Zacatecas (Figs. 2, 3 y 5).

El nombre genérico hace alusión al característico color verde oscuro que presentan las flores de las especies del grupo.

Clave para la identificación de las secciones y de las especies del género *Viridantha*

1 Rosetas regulares, más o menos esféricas en contorno, acaules o, si son caulescentes, el tallo de más de 1 cm de diámetro; hojas numerosas .... *Viridantha* sección *Viridantha*
  2 Inflorescencia escaposa, generalmente más larga que las hojas.
    3 Inflorescencia compuesta, globosa .... *V. plumosa*
    3 Inflorescencia simple, raramente biespigada, aplanada .... *V. ignesiae*
  2 Inflorescencia nidular, siempre más corta que las hojas.
    4 Hojas de menos de 2 mm de ancho; anteras amarillas; plantas generalmente epifíticas .... *V. atroviridipetala*
    4 Hojas de más de 3.5 mm de ancho; anteras negras; plantas rupícolas .... *V. mauryana*
1 Rosetas irregulares, amorfas en contorno, caulescentes, el tallo de menos de 0.5 cm de diámetro; hojas pocas .... *Viridantha* sección *Caulescens*
  5 Inflorescencia escaposa, generalmente más larga que las hojas, el pedúnculo de menos de 2 mm de diámetro .... *V. tortilis*
  5 Inflorescencia nidular o, si es escaposa, con el pedúnculo de más de 2.5 mm de diámetro y siempre más corta que las hojas .... *V. lepidosepala*

Viridantha sectio **Viridantha**

Herbae acaules, foliis numerosis, rosulatis, inflorescentia composita vel rarius simplici.

Viridantha atroviridipetala (Matuda) Espejo, comb. nov. Basiónimo: *Tillandsia atroviridipetala* Matuda, Cact. Suc. Mex. 2: 53-54, f. 40. 1957. TIPO: Estado de México,

Puente de Calderón, en orilla de arroyo, epífita en *Taxodium mucronatum*, 17.X.1955, *E. Matuda 32632* (Holotipo: MEXU (probablemente no existe ejemplar)). Fig. 1.

De acuerdo con el protólogo, el holotipo debería estar depositado en el Herbario Nacional (MEXU); sin embargo, no fue encontrado aun después de una revisión exhaustiva de la colección de bromeliáceas, por lo que es muy probable que no exista ejemplar alguno. Tampoco se sabe de la existencia de isotipos, al menos en los principales herbarios mexicanos (CHAPA, ENCB, IBUG, IEB, UAMIZ, XAL) y norteamericanos (F, HUH, NY, US). De igual modo, ha sido imposible localizar el paratipo (*Matuda 32633*) citado en la publicación original.

Por otra parte, existen dos duplicados del ejemplar *E. Matuda 30414*, recolectado en la localidad tipo, depositados en el Herbario Nacional (MEXU 128965!, MEXU 141734!). Cabe señalar que el Dr. Matuda tenía una manera muy peculiar de trabajar su material y como es sabido, existen innumerables inconsistencias en los datos, la numeración y la ubicación física de sus especímenes designados como tipos.

Debido a lo anterior y de acuerdo con el artículo 9, incisos 9.1 y 9.9 del Código Internacional de Nomenclatura Botánica, se propone aquí el siguiente neotipo: Hidalgo, 5 km al E de Metzquititlán, *J. Rzedowski 19504* (Neotipo (aquí designado): ENCB!; Isoneotipos: MEXU!, MICH!)

V. atroviridipetala se conoce de los estados de Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Oaxaca, Puebla y Zacatecas. Fig. 2.

Viridantha ignesiae (Mez) Espejo, comb. nov. Basiónimo: *Tillandsia ignesiae* Mez, Bull. Herb. Boissier sér. 2. 3: 143. 1903. TIPO: Michoacán, municipio La Huacana, Monte de Santa Inés "Ignes" [ca. 6-7 km NE de Inguarán] (fleurs) et de Las Cieneguillas "Seneguías" (fruits), 1500 m, 4.IV.1898, *E. Langlassé 93* (fleurs) (Holotipo: B ?; Isotipos: G!, GH!, K, P!, P, US 385728!).

Tillandsia lecomtei Poiss. & P. Menet, Bull. Mus. Hist. Nat. (Paris) 14: 236-237. 1908, *nomen superfluum*. TIPO: Michoacán, municipio La Huacana, Monte de Santa Inés "Ignes" [ca. 6-7 km NE de Inguarán] (fleurs) et de Las Cieneguillas "Seneguías" (fruits), 1500 m, 4.IV.1898, *E. Langlassé 93* (Holotipo: P!; Isotipos: B?, G!, GH!, K, P, US 385728!).

V. ignesiae se conoce de los estados de Guerrero, Jalisco, México y Michoacán. Fig. 2.

Viridantha mauryana (L. B. Sm.) Espejo, comb. nov. Basiónimo: *Tillandsia mauryana* L. B. Sm., Contr. Gray Herb. 117: 31, t. 2, f. 32, 33. 1937. TIPO: Hidalgo, cañada de Metztitlán, 1300-1500 m, 17.IV.1891, *P. Maury 5747* (Holotipo: GH!; Isotipos: F, GH!).

Existen en el Gray Herbarium (GH) dos duplicados de la colecta de Maury, ambos anotados por Smith. El holotipo, sin embargo, tiene escrita la palabra "TYPE", por lo que no hay lugar a dudas sobre cuál espécimen es el holotipo y cuál es el duplicado.

V. mauryana se conoce de los estados de Hidalgo, Jalisco, Oaxaca y Zacatecas. Fig. 3.

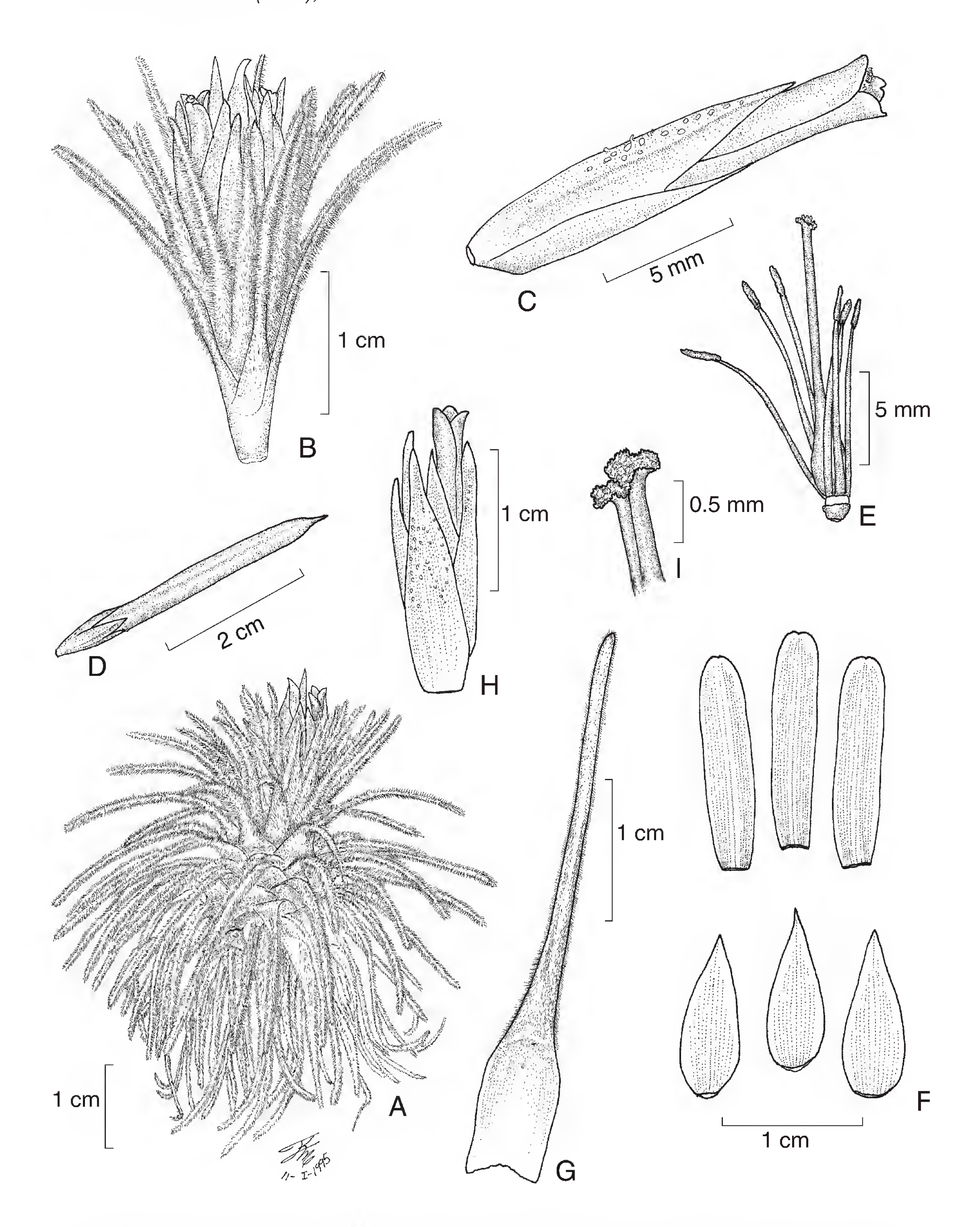


Fig. 1. *Viridantha atroviridipetala* (Matuda) Espejo. A. Hábito; B. Inflorescencia; C. Flor; D. Fruto; E. Detalle del androceo y el gineceo; F. Pétalos y sépalos; G. Hoja; H. Detalle de una espiga; I. Estigma.

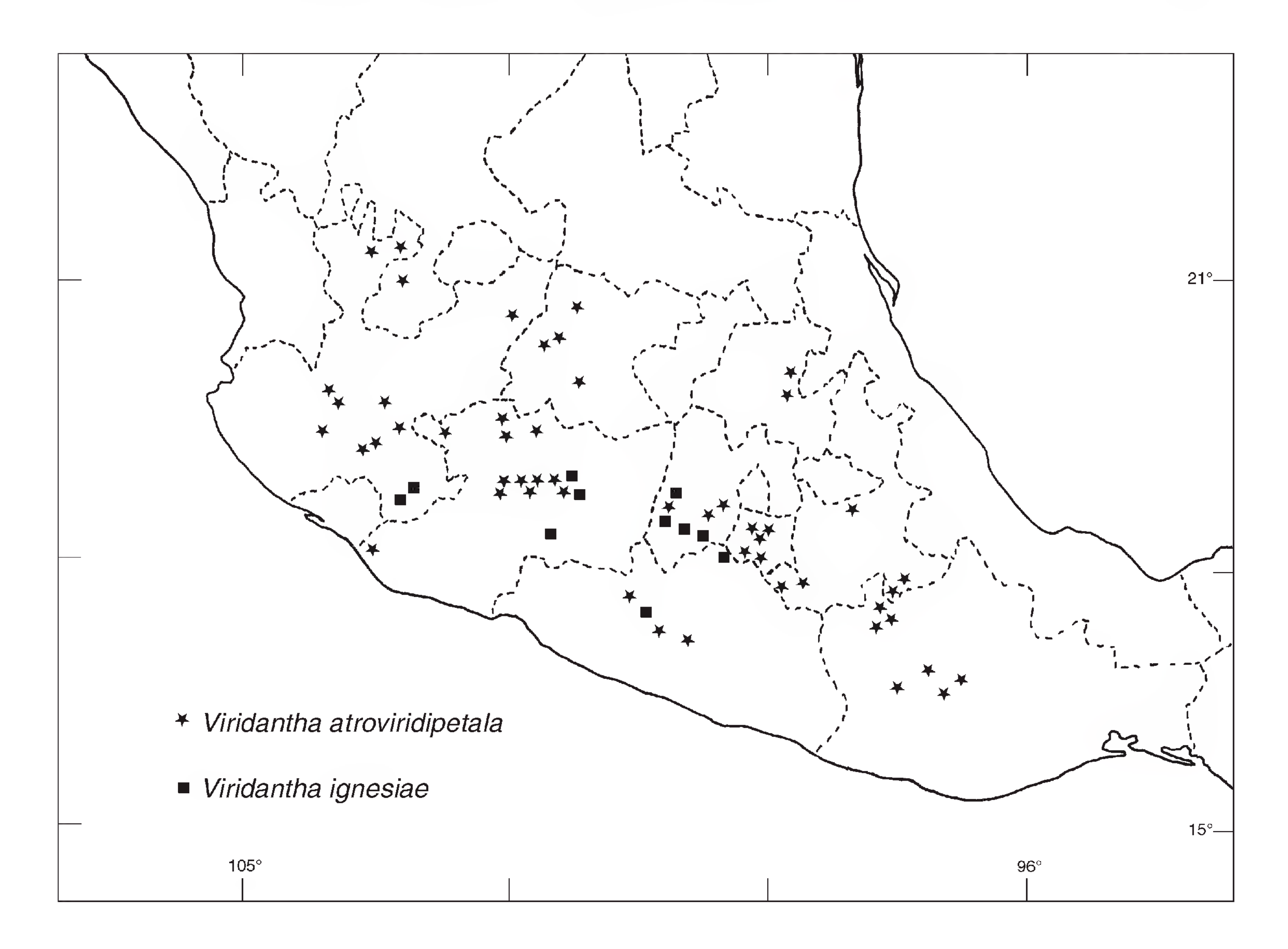


Fig. 2. Distribución conocida de *Viridantha atroviridipetala* (Matuda) Espejo y *V. ignesiae* (Mez) Espejo.

Viridantha plumosa (Baker) Espejo, comb. nov. Basiónimo: *Tillandsia plumosa* Baker, J. Bot. 26: 13. 1888. TIPO: Puebla, *G. Andrieux 57* (Holotipo: K foto!; Isotipo: M).

V. plumosa se conoce de los estados de Guerrero, México, Oaxaca y Puebla. Fig. 5.

Viridantha sectio **Caulescens** Espejo, sectio nova

Differt a *Viridantha* sectione *Viridantha* caule elongato, foliis paucioribus et inflorescentia simplici.

TYPUS: *Viridantha tortilis* (Klotzsch ex Baker) Espejo

Viridantha lepidosepala (L. B. Sm.) Espejo, comb. nov. Basiónimo: *Tillandsia lepidosepala* L. B. Sm., Proc. Amer. Acad. Arts 70 (Contr. Gray Herb. 106): 155, t. 2, f. 2,

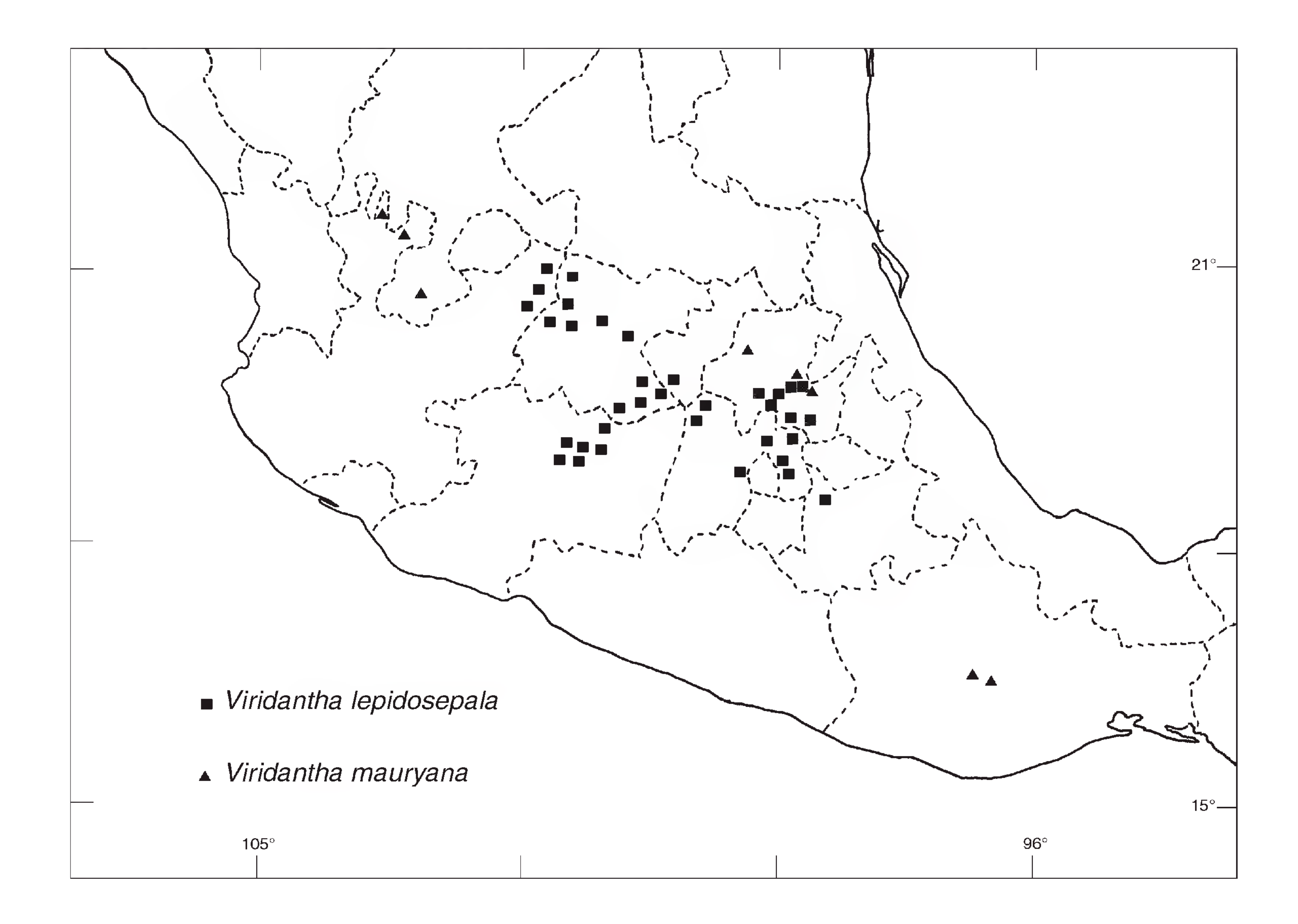


Fig. 3. Distribución conocida de *Viridantha lepidosepala* (L. B. Sm.) Espejo y *V. mauryana* (L. B. Sm.) Espejo.

3. 1935. TIPO: Michoacán, on trees near Lake Cuitzeo, 9.VIII.1892, *C. G. Pringle 5323* (Holotipo: GH!; Isotipo: VT!).

V. lepidosepala se conoce de los estados de Guanajuato, Hidalgo, Jalisco, México, Michoacán, Puebla y Querétaro. Fig. 3.

Viridantha tortilis (Klotzsch ex Baker) Espejo, comb. nov. Basiónimo: *Tillandsia tortilis* Klotzsch ex Baker, J. Bot. (London) 25: 237. 1887, non Brongn. ex E. Morren, 1878, nomen. TIPO: San Luis Potosí, Central Mexico, chiefly in the region of San Luis Potosí, 22° N, 6000-8000 ft [1800-2400 m], 1878, *C. C. Parry & E. Palmer 872* (Lectotipo (designado por Till, 1986): BM; Isolectotipos: E, GH!, K, P!, US). Fig. 4.

Tillandsia ehrenbergii Klotzsch ex Beer, Fam. Bromel. 264. 1857, nomen nudum. *Platystachys ehrenbergii* Beer, Fam. Bromel. 264. 1857, *pro syn.* TIPO: Hidalgo, prope

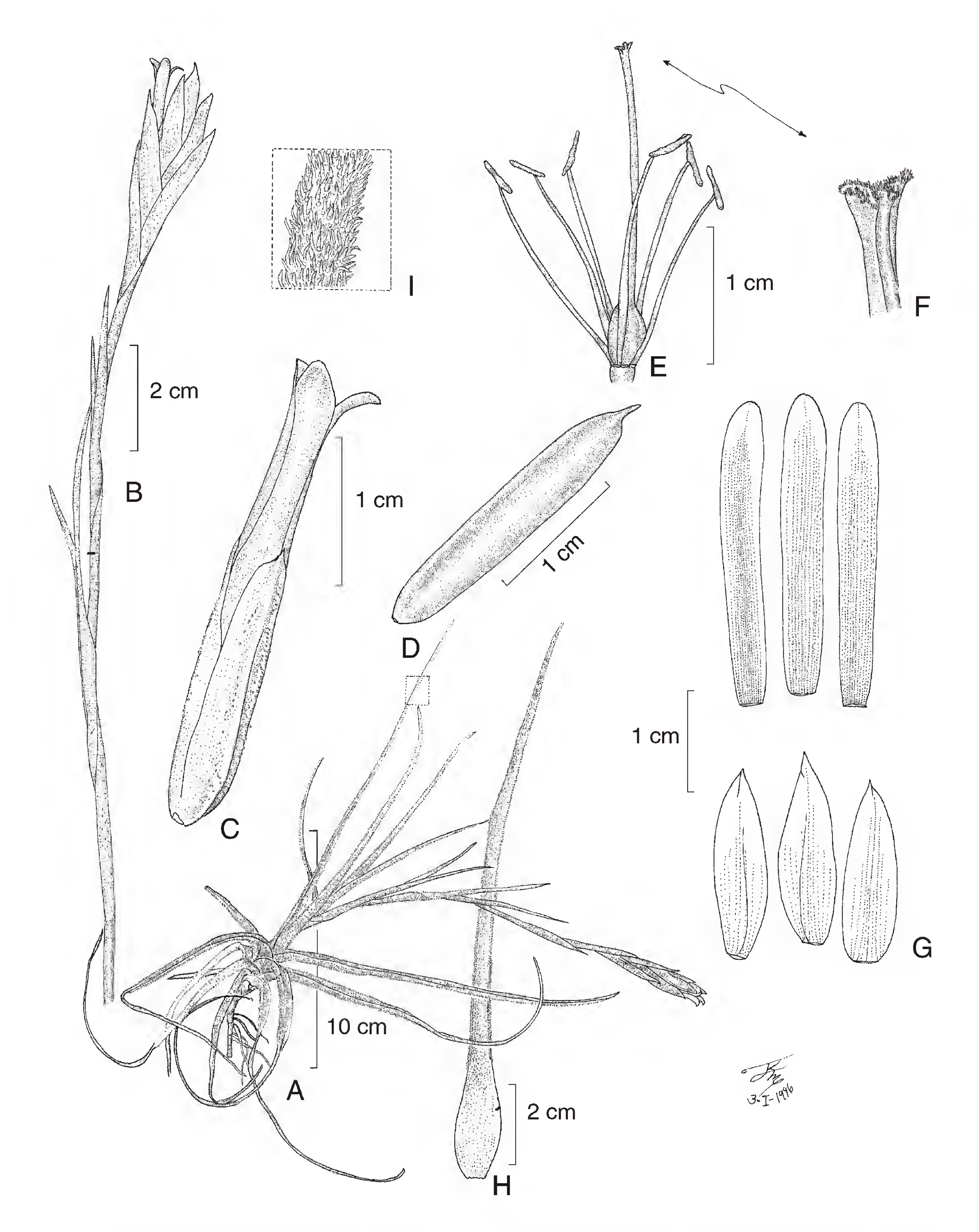


Fig. 4. *Viridantha tortilis* (Klotzsch ex Baker) Espejo. A. Hábito; B. Inflorescencia; C. Flor; D. Fruto; E. Detalle del androceo y el gineceo; F. Estigma; G. Pétalos y sépalos; H. Hoja; I. Detalle de escamas en la hoja.

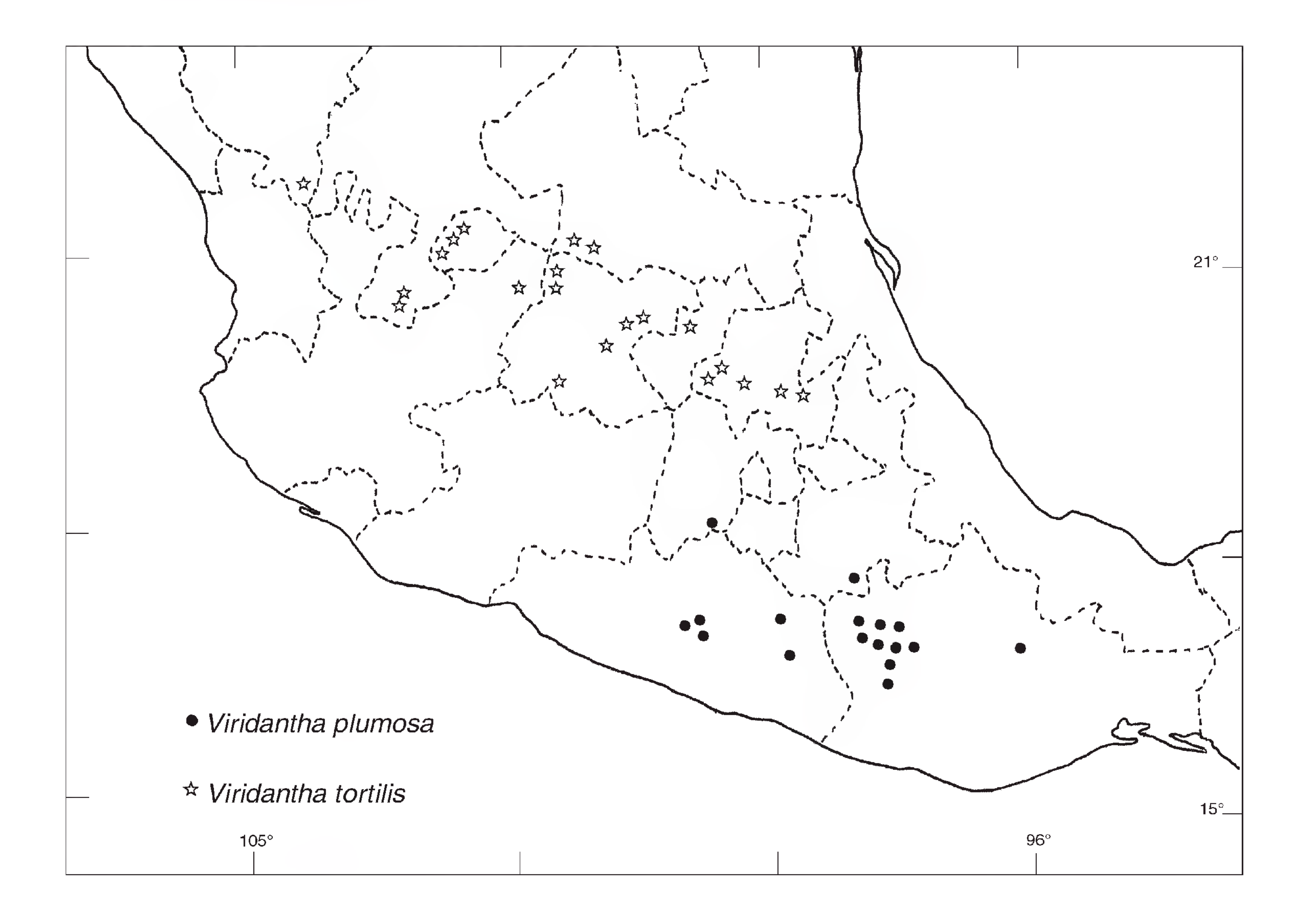


Fig. 5. Distribución conocida de *Viridantha plumosa* (Baker) Espejo y *V. tortilis* (Klotzsch ex Baker) Espejo.

[San Miguel] Regla "Rugla", *C. Ehrenberg 860* (Holotipo: B?; Isotipos: GH!, MEXU 7636!, MEXU 169260!, VT!).

Tillandsia tortilis Klotzsch ex Beer, Fam. Bromel. 266. 1857, nomen nudum. *Platystachys tortile* Beer, Fam. Bromel. 266. 1857, pro syn., nomen nudum. TIPO: ?

Tillandsia ehrenbergiana Klotzsch ex Baker, Handb. Bromel. 169. 1889, pro parte, nomen superfluum (*C. C. Parry & Palmer 872*).

Tillandsia ehrenbergii Mez, in A. DC., Monogr. Phan. 9: 727. 1896, pro parte, nomen superfluum (*C. C. Parry & Palmer 872*).

Tillandsia tortilis Klotzsch ex Baker ssp. *curvifolia* Ehlers & Rauh, J. Bromeliad Soc. 40: 167-168, f. 7, 8. 1990. TIPO: Guanajuato, apud Guanajuato, 2200-2400 m, 6.III.1985, *R. & K. Ehlers M 850201* (Holotipo: WU).

V. tortilis se conoce de los estados de Aguascalientes, Durango, Guanajuato, Hidalgo, Jalisco, Querétaro, San Luis Potosí y Zacatecas. Fig. 5.

Esta especie, con una complicada historia taxonómico nomenclatural, fue durante largo tiempo conocida como *Tillandsia ehrenbergii*, nombre con el cual se encontraba

identificada en la mayoría del material de herbario revisado. Sin embargo, Till (1986) aclaró la identidad del taxon, demostrando que el nombre correcto para el mismo debe ser *T. tortilis*.

AGRADECIMIENTOS

Quiero expresar mi gratitud a Ana Rosa López-Ferrari por su apoyo en el desarrollo del trabajo. Jerzy Rzedowski, Graciela Calderón de Rzedowski, Jacqueline Ceja, Walter Till y Sue Sill leyeron el manuscrito y lo enriquecieron con sus comentarios. Fernando Chiang revisó el artículo, en especial las cuestiones nomenclaturales y el latín. Las ilustraciones fueron realizadas por Rolando Jiménez Machorro. A los curadores de los herbarios revisados, agradezco las facilidades que me otorgaron para consultar las colecciones o, en su caso, el préstamo del material tipo.

Los resultados de este trabajo forman parte del proyecto de tesis de doctorado del autor.

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Recibido en marzo de 2002.
Aceptado en septiembre de 2002.

Acta Botanica Mexicana (2002), 60: 37-77

HIGHER PLANT VACUOLAR IONIC TRANSPORT IN THE CELLULAR CONTEXT

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ABSTRACT

The vacuole, occupying up to 95% of the volume of a mature higher plant cell, serves as a main store of solutes and as the key element of intracellular Ca^{2+}- and pH-stat. The role of the vacuole in the ionic homeostasis of the cell is also evident under K^+-starving conditions and under salt stress. It is involved in the regulation of turgor and cell volume, and in specialized cells, such as stomatal guard cells, a large central vacuole is a principle component of the cell osmotic motor. For more than a decade, the guard cell has gained the status of a higher plant cell model, intensely studied by physiological, cell and molecular biology techniques. Despite tremendous progress in the understanding of signal transduction events taking place at plasma membrane of guard cells, the involvement of vacuolar ion transporters remains a significant challenge. Like in a plasma membrane, the ionic transport across the vacuolar membrane, the tonoplast, is directed and coordinated through a complex set of specific transport proteins, including pumps, transporters, and ionic channels. During recent years, the application of modern electrophysiological techniques (principally, the patch-clamp) has enabled numerous individual ion channels to be functionally characterized, although none of them has been structurally identified. The work of our group is focused on the characterization of so called slow (SV) and fast (FV) vacuolar ion channels, which have a wide, if not ubiquitous, distribution in higher plants, and can be detected in stems, leaves, as well as in root storage tissue. Their ionic selectivity, gating by membrane voltage and by physiologically abundant cations, as well as their possible roles in signal transduction and ionic balance are discussed.

Key words: intracellular calcium, ion channels, ionic transport and homeostasis, patch-clamp, polyamines, signal transduction, vacuole.

RESUMEN

La vacuola ocupa hasta 95% del volumen celular en las plantas superiores, es un reservorio de solutos y un organelo clave en el control de la concentración de Ca^{2+} y del pH intracelulares. El papel de la vacuola en la homeostasis iónica celular es también evidente en condiciones ambientales de falta de potasio y estrés salino. La vacuola central está involucrada en la regulación de la turgencia y el volumen celular en células especializadas como las estomáticas, constituyendo

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el principal componente del motor osmótico celular. Desde hace más de una década, la célula estomática ha ganado un status de modelo celular en plantas superiores, siendo extensamente estudiada mediante técnicas fisiológicas y de biología molecular y celular. A pesar del gran avance en el conocimiento de la transducción de señales en la membrana plasmática, el entendimiento de los mecanismos implicados en el transporte iónico en la membrana vacuolar continúa siendo un gran reto. Al igual que en la membrana plasmática, el transporte iónico en la membrana vacuolar o del tonoplasto, está dirigido y coordinado a través de un complejo sistema integrado por proteínas de transporte que incluye bombas (H^+-ATPasa, pirofosfatasa, bomba de Ca^{2+}), transportadores (antiportadores Na^+/H^+ y Ca^{2+}/H^+) y varios canales iónicos. En años recientes, con la aplicación de las técnicas modernas de electrofisiología (patch clamp), numerosos canales iónicos han sido caracterizados funcionalmente, aunque ninguno de ellos ha sido estructuralmente identificado. El trabajo de nuestro grupo se ha enfocado a la caracterización de los canales iónicos vacuolares lentos (SV) y los canales iónicos vacuolares rápidos (FV); estos canales tienen una amplia, si no es que ubicua, distribución en plantas superiores, se les encuentra en células troncales, en células diferenciadas de las hojas o de los tejidos de almacenamiento de las raíces. En este trabajo se discuten las características funcionales de estos canales en cuanto a selectividad iónica, mecanismos de compuerta dependientes de voltaje e influencia de cationes de importancia fisiológica (calcio, magnesio, protones, poliaminas), así como su posible papel fisiológico. Asimismo, se plantean los principales temas que están por resolverse. Particularmente, se analiza la participación de los canales aniónicos en la función de la vacuola como motor osmótico. Se sabe que el tonoplasto presenta una gran permeabilidad para varios aniones, sin embargo, los canales iónicos involucrados no han sido claramente identificados. El otro problema es la identificación de los canales activados por ligandos intracelulares. A pesar de que las células vegetales poseen receptores intracelulares para inositol 1,4,5 trifosfato (IP_3) y adenosinadifosfatoribosa cíclica (cADPR), su ubicación celular no está definida. Por el momento no son confiables las evidencias que indican la existencia de canales iónicos vacuolares activados por estos factores.

Palabras clave: calcio intracelular, canales iónicos, transporte y homeostasis iónica, patch-clamp, poliaminas, transducción de las señales, vacuola,

VACUOLE, IONIC HOMEOSTASIS AND OSMOTIC MOTORS OF PLANT CELLS

Vacuole and ionic homeostasis. The vacuole controls cell volume and turgor pressure of a plant cell, plays a key role in the regulation of cytosolic Ca^{2+} and pH, serves as a dynamic store of sugars, amino acids and nutrients, and sequesters xenobiotics and toxic ions (e.g., heavy metals). The ion transport ability of the vacuolar membrane, the tonoplast, controls all these functions, either directly, via release or uptake of certain ions, or indirectly, by setting the membrane voltage and H^+ gradient to define the direction of H^+-coupled secondary transport of non-ionic compounds.

The vacuole is the central storage compartment of a higher plant cell, and, as such, is used to deposit nutrient ions, as for instance nitrate (Zhen & Leigh, 1990), inorganic phosphate (Bielski, 1968), and potassium (Walker et al., 1996). In plants with crassulacean acid metabolism (CAM), the only way for the temporal storage of CO_2 is in the form of organic acids (malate) in the vacuole (Cheffings et al., 1997; Raven, 1997).

There is a well-defined example of K^+ homeostasis in a vacuolated plant cell (Leigh & Wyn Jones, 1984; Walker et al., 1996). Vacuolar K^+ is thought to play a sheer osmotic role; cytosolic K^+, besides this, regulates the activity of several enzymes, particularly, of those involved in the protein synthesis (Evans & Sorger, 1966). Therefore, K^+ concentration

in the cytosol needs to be maintained at about the 100 mM level. At K^+ deficiency, K^+ in the cytosol is kept fairly constant at the expense of the vacuolar K^+ until the latter reaches a critical level ~20 mM, and only at this point the decline in cytosolic K^+ is initiated (Walker et al., 1996). Similarly, when the extracellular supply of NO_3^- is limited, the cytosolic NO_3^- pool is refilled by NO_3^- stored in the vacuole (Zhen & Leigh, 1990). In barley roots, cytosolic nitrate is kept around 4 mM, whereas vacuolar NO_3^- shows a large variation due to the accumulation of nitrate or its mobilization at nitrate-limiting conditions (Van der Leij et al., 1998). Phosphate in maize root cells also behaves in a similar fashion: whereas in cytosol its concentration is independent of nutritional status and kept in the range 4-6 mM, in vacuoles of phosphate-replete plants, it is between 4 and 20 mM, and under phosphate starvation it is vanishing low (Lee et al., 1990).

Whilst the aforementioned examples deal with essential nutrients, whose concentration in cytosol needs to be kept at a certain level regardless of the growing conditions, another example of ionic homeostasis, based on the vacuolar transport capacity, is provided by a plant's response to high salinity. Excess Na^+ in the cytosol is toxic, so external Na^+ entering the cytosol through cationic channels needs to be extruded from the cell (Shi et al., 2000) or sequestered into the vacuole. Extreme halophytes, such as the ice plant, *Mesembryanthemum crystallinum*, can accumulate up to 1 M of Na^+ in the vacuole (Adams et al., 1992). This is possible via the function of the Na^+/H^+ antiporter of the vacuolar membrane energized by the H^+ pumping V-ATPase. Both, the Na^+/H^+ antiporter and the H^+ -ATPase are known to be stimulated by salt stress (Barkla et al., 1995; Barkla & Pantoja, 1996; Bethke & Jones, 2000; Ratajczak, 2000).

Finally, the vacuole plays a pivotal role in H^+ and Ca^{2+} homeostasis. Due to its large size, the vacuole serves as a main sink for these ions in the plant cell. Whereas cytosolic pH is normally maintained in the 7-7.5 range, vacuolar pH of 5 to 5.5 is typical, and in acidic fruits and in leaves, accumulating oxalic acid, vacuolar pH as low as 2.7 may be observed (Smith & Raven, 1976). The resting Ca^{2+} activity in the cytosol of most living cells is in the range of 100-300 nM. An increase of free calcium up to 500 nM-1 μM serves as a trigger, firing a cascade of signal transduction events. The vacuolar free Ca^{2+}, as reported by ion-selective microelectrodes, is in the range of 0.2-2 mM (Bethmann et al., 1995; Allen & Sanders, 1997). As the transtonoplast electrical potential is close to zero (+5- -20 mV), this implies that huge electrochemical gradients, 100-fold for H^+ and 10^3 -10^4 fold for Ca^{2+}, are therefore actively generated. K^+ seems to be distributed almost equally between the cytosol and the vacuole (Bethmann et al., 1995). Thus, it can be driven passively into and out of the vacuole, depending on the nutrient supply for this ion. For inorganic anions (Cl^- and NO_3^-), a moderate 3-10 fold cytosol-directed concentration gradient was detected. Phosphate is distributed evenly, and malate may be concentrated in the vacuole up to the 100 mM level, as compared to a few millimolar in the cytosol (Bethmann et al., 1995; Allen & Sanders, 1997; Barbier-Brygoo et al., 2000). A summary of transtonoplast electrochemical gradients for main inorganic ions is presented in Fig. 1. In conclusion, it should be noted that despite existing transtonoplast ionic gradients, the vacuole is at any moment in osmotic equilibrium with the cytosol due to the extremely high water conductivity of the tonoplast (Maurel et al., 1997; Kjellbom et al., 1999).

Generation of turgor pressure. Functional morphology and anatomy, especially in non-woody plants, may not be maintained without generation of turgor pressure in every

plant cell. This forms a basis of the hydrostatic skeleton of the whole plant. The cell becomes turgid due to the accumulation of solutes in its large central vacuole. This creates a driving force for the influx of water, whereas the resulting volume expansion is delimited by the rigidity of the cell wall. On the energetic costs grounds, it is much more efficient to accumulate inorganic ions in the vacuole via H^+-coupled transport than to synthesize organic compounds de novo to be used as a pure osmoticum (Raven, 1997). However, for stomatal guard cells this consideration is valid only under the energy (solar light) deficient conditions that occur in the morning, whereas during high photosynthetic activity in the afternoon, potassium salts in the vacuolar solution are almost completely substituted by sucrose (Amodeo et al., 1996; Talbott & Zeiger, 1996). Another advantage of turgor generation via ion uptake is the possibility to release turgor pressure through high-capacity passive ion transport pathways: ion channels. Indeed, ion channels can rapidly transfer small inorganic ions but generally not organic compounds. The latter are transported instead by means of at least 1,000-fold-slower carriers. Thus, fast (in seconds to minutes) volume changes may occur in specialized cells, such as pulvinar motor cells guiding leaves movements (e.g., *Mimosa pudica*) but also, and this is of general importance for all land plants, in stomatal guard cells.

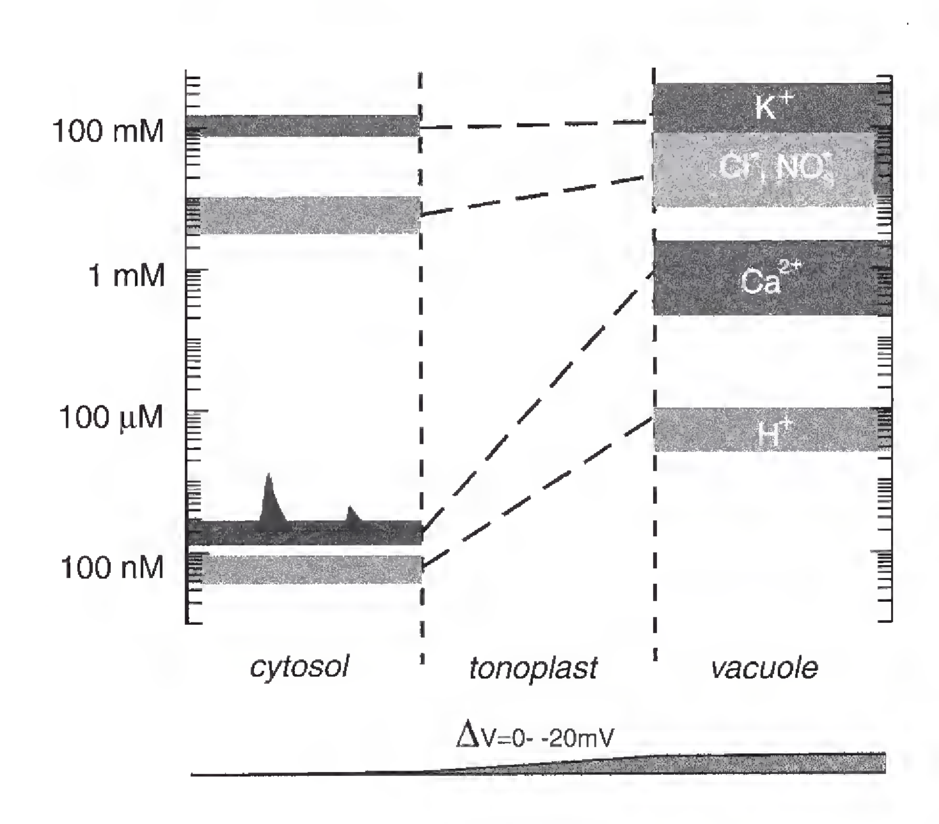


Fig. 1. Electrical and chemical gradients for main inorganic ions across the vacuolar membrane (tonoplast). Values are expressed as activities. Shadowed areas correspond to reported concentration ranges for different species (Allen & Sanders, 1997; Barbier-Brygoo et al., 2000) and dashed lines connect mean values for the cytosol and vacuole, respectively. Cytosolic Ca^{2+} resting range is indicated, whereas Ca^{2+} activity (free concentration) spikes can be observed. Special instances of very acidic pH in vacuoles, e.g., of immature fruits, are not shown. The transtonoplast electrical potential convention is according to Bertl et al. (1992), cytosol minus vacuole ($+V=V_{cyt}-V_{vac}$). Thus, a negative potential favors the release of cations from the vacuole and uptake of anions, respectively.

Function of the stomata. Stomata are pores formed by two bean-shaped cells, located primarily on the lower leaf epidermis. They serve as main gates for water and gas (CO_2) exchange with the environment. Performance of this function is of vital importance for the two main physiological functions of plants, photosynthesis and transpiration. Stomatal complexes can be traced in early land plants down to the Silurian period, ca 400 millions years ago (Edwards et al., 1998). There are no significant differences between the morphology of fossil stomata and those of extant plants, though stomatal density has increased since ancient times due to a decrease in the atmospheric CO_2 concentration (Beerling et al., 1998; Edwards et al., 1998). Due to a unique radial arrangement of actin filaments in the cytoskeleton of the guard cell, turgor pressure changes drive cell volume modifications, which in turn are transformed into changes in size of the stomatal aperture (Kim et al., 1995). Stomatal closure is associated with a decrease of volume, cell shrinkage, and, occasionally, with the fragmentation of a large central vacuole. There are no plasmodesmal contacts between adjacent cells in mature stomatal complexes (Wilmer & Fricker, 1996). Guard cells are quite resistant to acid treatment, so it is possible therefore to analyze stomatal movements *in situ*, in epidermis strips or even in whole leaves, by killing cells other than the guard cells. The conventional voltage-clamp, as well as ion-selective microelectrode techniques, tracer flux measurements, and ratiometric fluorescence measurements, may be then applied to study ionic fluxes and concentration changes. On the other hand, guard cell protoplasts, and further, vacuoles, may be easily isolated and utilized for patch-clamp recordings of the activity of individual ion transport proteins (Raschke & Hedrich, 1989). Therefore, guard cells serve as an excellent experimental model to study the ion basis of signal transduction in plants. Stomatal opening is elicited by blue light, auxin, and some fungal toxins (fusicoccin), whereas stomatal closure is induced by high CO_2, darkness, oxidative stress, abscisic acid (ABA) and water deficiency, among other factors (McAinsh et al., 1997; Assmann & Shimazaki, 1999). The closing and opening of stomata is accompanied by massive changes in ionic contents. Concentration of K^+ and Cl^- in open stomata are ~450 mM and ~120 mM, whereas in closed ones these concentrations are ~100 mM and ~30 mM, respectively (Penny & Bowling, 1974; Penny et al., 1976). Every 1 μm change of stomata aperture diameter is associated with an approximately 30 mM change in K^+ content (Wilmer & Fricker, 1996). The difference in volume between open and closed states of a *Vicia faba* guard cell is 200-300%; the osmotic potential changes by 200-300 mOsm; and ~2 pmole of KCl is released over a period of 30 min (closing) or is taken up in 2-3 h (opening) (Blatt, 2000). Even for the relatively rapid process of stomatal closure, a cell must maintain a K^+ and Cl^- release across vacuolar and plasma membranes of just ~100 pA in each guard cell, which translates to few tens of open K^+ and anion channels. In recent years a number of excellent reviews have appeared devoted to the regulation and co-ordination of the ion transporter function during stomatal response (MacRobbie, 1998; Grabov & Blatt, 1998; Pei et al., 1998; Assmann & Schimazaki, 1999; Blatt, 2000; Schroeder et al., 2001). In this paper we will feature only some mechanisms leading to stomatal closure in response to the application of ABA, the "drought-hormone" (Fig. 2). Ion transport across the plasma membrane of a guard cell is well characterized. It is mediated by a handful of key transporters, including two K^+-selective channels (one mediating K^+ influx (K_{in}) and another K^+ efflux (K_{out})), the slow anion channel, the Ca^{2+}-influx channel, and the vanadate-sensitive H^+ ATPase (Fig. 2). However, our understanding of the function of the tonoplast

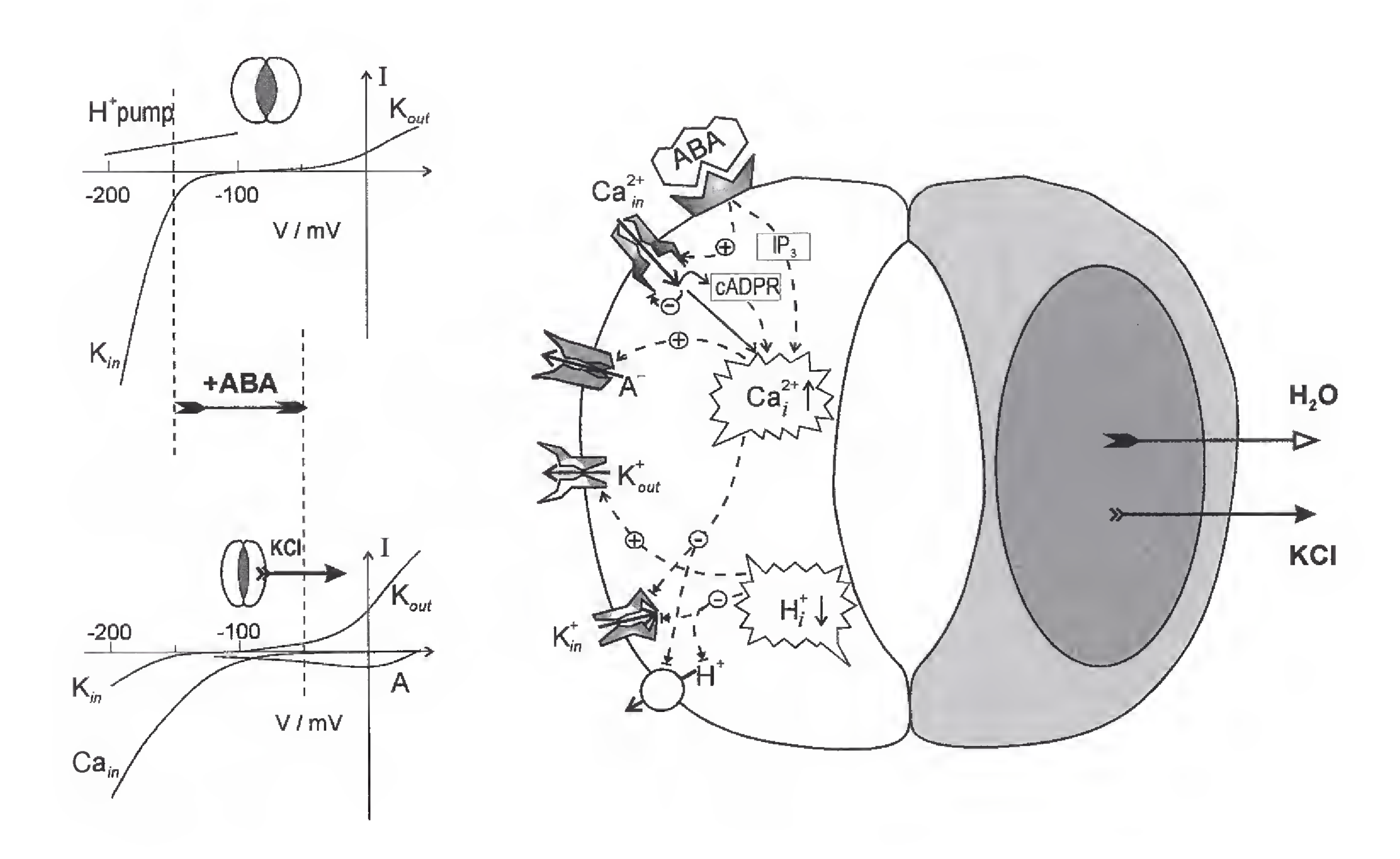


Fig. 2. ABA-induced stomatal closure: electrocoupling of ionic currents and communication via intracellular Ca^{2+} and pH trigger KCl loss across the guard cell membrane. Left: whole cell ionic currents before and after addition of ABA. Switch of membrane potential level from KCl uptake conditions to the conditions of net KCl loss in response to ABA is indicated by an arrow between two dashed lines. Relative amplitudes of ionic currents carried by H^+-pump, inwardly rectifying K^+ channels (K_{in}), outwardly rectifying K^+ channels (K_{out}), slow anion channels (A), and Ca^{2+}-influx channels (Ca_{in}) are collected from the original measurements in *Vicia faba* guard cell membrane by Blatt (1987), Schroeder & Keller (1992), Hamilton et al. (2000), Grabov & Blatt (1997,1999). For simplicity, it is assumed that anion channels are not active, and the H^+ pump is only active at resting Ca^{2+}_i. Right: ABA binds to an unidentified receptor and activates the Ca^{2+} influx channel and Ca^{2+} release from IP_3-sensitive intracellular stores, possibly via a G-protein-phospholipase C pathway (Blatt, 2000). A rise in intracellular Ca^{2+} is amplified and prolonged by the activation of Ca^{2+} release through cADPR-receptor channels. Independently, ABA causes the alkalization of the cytosol. A decrease in H^+_i and an increase in Ca^{2+}_i concentration down-regulates inward K^+ channels and H^+-ATPase (Kinoshita et al., 1995; Grabov & Blatt, 1999), whereas outward K^+ channels are stimulated by alkaline pH_i (Grabov & Blatt, 1997). An increase in Ca^{2+}_i causes the activation of anion channels, which, together with outward rectifier K^+ channels, causes membrane depolarization, thus a release of KCl from the cell is promoted. The reversibility of this process is secured by inactivation of Ca^{2+} channels due to increase of cytosolic Ca^{2+} and membrane depolarization; ABA may evoke several cycles (oscillations) of Ca^{2+} channels activity (Hamilton et al., 2000). Ion fluxes are shown by solid arrows, modulating by dashed arrows, positive and negative regulation are indicated by (+) and (-), respectively.

ion channels and the means of their regulation is much less satisfactory. Based on tracer flux measurements, up to 90% of K^+ salts lost during stomatal closure come from the vacuole (MacRobbie, 1998). Therefore, some yet non-identified vacuolar K^+- and anion-release channels must operate in conjuction with their plasma membrane counterparts. Meanwhile, some specific hypotheses involving vacuolar K^+ and Ca^{2+}-permeable channels have been generated (e.g., Ward & Schroeder, 1994) and will be further discussed.

ROUTES OF THE IONIC TRANSPORT ACROSS THE TONOPLAST

General features of transporters. Ions can not pass through biological membranes via simple diffusion and always require a specialized transporter. Transporters are classified into three categories: primary pumps, carriers (porters), and channels. The transport of H^+ the vacuolar membrane by primary pumps is coupled to the hydrolysis of high-energy compounds, ATP (by an ATPase) and inorganic pyrophosphate (by a PPase). Carriers can transport ions either passively, down the electrochemical gradient (all uniporters), or utilize the gradient of one ion to transport another one, even against the electrochemical gradient of the latter (co-transporters). Tonoplast carriers, e.g., the Na^+/H^+ antiporter, generally utilize a H^+ gradient created by H^+ pumps[1]. Ion channels, when open, are water-filled pores which transport ions exclusively downhill with respect to their electrochemical gradients. The difference between ion channels and uniporters is their capacity as well as their mechanism of conduction. However, in the absence of a specific substrate, the carrier may operate as a high-conductance ion channel. An example of such a phenomonan reported for plant systems was the conversion of the chloroplast's inner membrane triose phosphate/ phosphate translocator into a high-conductance anion channel upon replacement of natural substrates by Cl^- (Schwarz et al., 1994). In general terms, a passive ion transporter may be represented as an ion channel with a switch inside, which binds one ion at any one moment and translocates it from one side of the membrane to the other. The conformational transition of the switch, if occurring in a "true" ion channel, needs to be not rate-limiting, i.e., faster than or comparable to the diffusion of the ion, although in certain occasions it is supposed to be the case (Hansen et al., 1997; Allen et al., 1998b). Therefore, ionic channels, normally operating a few tens of millivolts away from their equilibrium potential, may generate currents in pA-range, where 1 pA is equivalent to $6x10^6$ ions/s. In comparison, the maximum velocity of 250 Na^+ ions/sec can be estimated for the red beet vacuolar Na^+/H^+ antiporter from the data of Barkla et al. (1990), whereas V-ATPases transport 60-180 H^+/sec assuming a $2H^+$/ATP stoichiometry (Ratajczak, 2000). In general, channels are responsible for a rapid, low-affinity ion transport, whereas carriers and pumps mediate a low-rate, high-affinity ion transport. The latter is particularly important when the substrate, for instance H^+, naturally occurs at low concentrations. Although V-ATPase is highly abundant, reaching 6.5-35% of the total tonoplast protein (Ratajczak, 2000), within physiological range of the transtonoplast

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[1] For the sake of completeness, it should be noted that secondary compounds, such as glutathione-conjugates, gluconurides, bilic acids, catabolites of chlorophyll, sulfated or sulfonated compounds, as well as some glycosides, are transported into vacuoles by means of direct energization (ATP-hydrolysis), mediated by so called ABC transporters (Martinoia et al., 2000).

potentials, the whole vacuole H^+-pump current reached only 10-30 pA at optimal conditions (Davies et al., 1994; Gambale et al., 1994). In other words, few open ion channels per vacuole may electrically balance the net H^+ current generated by the whole V-ATPase population. The total number of copies of the dominant ion channels in plant vacuoles ranges from 2,000 to 10,000 (Schulz-Lessdorf & Hedrich, 1995, Tikhonova et al., 1997, Pottosin et al., 1997). These numbers are comparable to the number of Na^+/H^+ antiporter units: 10,000 per vacuole (Barkla et al., 1990). Keeping in mind the huge difference in transport numbers between ion channels and porters, together with the low selectivity of vacuolar cation channels (see below) and the physiological demand to keep low Na^+ (1-10 µM) in the cytosol, the maintenance of about a 10-fold vacuole to cytosol Na^+ concentration gradient (Martinoia et al., 1986; Barkla et al., 1995) requires a tight control of the ion channel function. Permanent opening of a single non-selective cation channel per vacuole could in principle dissipate the Na^+ gradient across the tonoplast.

MEMBRANE ENERGIZATION BY H^+ PUMPS

The function of tonoplast H^+ pumps is to take up protons into the vacuole, thus contributing to the cytosol pH-stat and energizing the vacuolar membrane for secondary transports against electrochemical gradients for corresponding ions. It is not clear why the tonoplast possesses two H^+-translocation pumps: V-ATPase and PPase (inorganic pyrophosphatase). The possession of these two pumps is unique feature of vacuole among eucaryotic organelles (Bethke & Jones, 2000).

V-ATPase. Plant V-ATPase is found mainly in the vacuolar membrane, although in some occasions V-ATPase activity can be detected in endoplasmic reticulum, Golgi apparatus, coated vesicles, and provacuoles (Maeshima, 2001). V-ATPase is a multi- (up to 10) subunit enzyme, whose structure is closely related to the F-type ATPases and consists of head complex V_1, involved in ATP hydrolysis, and transmembrane proton-conducting channel V_0. In contrast to chloroplast and mitochondrial F-type ATPases, V-ATPase is insensitive to azide and oligomycin and can be specifically inhibited by submicromolar concentrations of concanamycin A and bafiolomycin (Ratajczak, 2000). V-ATPase is inhibited by nitrate and stimulated by Cl^- at their physiological range of concentrations, and stimulated by magnesium as a constituent of divalent cation-ATP complex with an apparent K_m between 0.2-0.8 mM (Lüttge & Ratajczak, 1997). It has been shown that the mutation of the *det3* encoding C subunit of V-ATPase in *Arabidopsis* results in the organ-specific alteration of growth due to restricted cell wall expansion, light-specific morphogenesis in the dark, and failure to close stomata in response to high external Ca^{2+} and H_2O_2 (Schumacher et al., 1999). It appears that the failure to close stomata is caused by a disruption of the specific Ca^{2+} oscillator depending on proton gradient generated by V-ATPase (Harper, 2001). Notably, various isoforms of C-subunit (e.g., four isoforms in *Arabidopsis*) show tissue-specific and developmentally regulated expression (Ratajczak, 2000). V-ATPase is not just a constitutive “house-keeping” enzyme, it is also an “eco-enzyme”, because under conditions of environmental stress, especially under salt stress, the subunit’s expression is enhanced and the holoenzyme structure is modified (Barkla & Pantoja, 1996; Ratajczak, 2000). Interestingly, in cultured carrot cells, salt stress is

followed by 20% decrease in ATP-hydrolisis activity of the V-ATPase, whereas the H^+ pumping rate is increased by 60% (Löv & Rausch, 1996). This implies an increased V-ATPase coupling rate, likely due to structural modification of the channel (V_o) domain (Ratajczak, 2000). The coupling rate, i.e., the H^+/ATP ratio, depends also on the pH gradient, changing from 3.2 to 1.75 upon an increase of) pH from ~3 to 4.75 units. This permits the acidification of the vacuolar lumen to pH<4 against a steep transmembrane gradient for protons (Davies et al., 1994). The expression of the V-ATPase subunit is up-regulated via promoters by phytohormones: ABA, ethylene, and possibly also jasmonic acid (Ratajczak, 2000).

PPase. Vacuolar H^+-translocating PPase is formed by a single polypeptide with a molecular mass about 80 kD. V-PPases are found in *Archaea*, *Eubacteria* (e.g., photosynthetic bacteria such as *R. rubrum*), protists (e.g., *Trypanasoma* and *Plasmodium*), green algae, and higher plants (Drozdowicz & Rea, 2001). Compared to V-ATPase, there is no obvious clue as to the exclusive physiological role of PPase. In contrast to V-ATPase, whose expression is approximately constant at different developmental stages, V-PPase is predominantly expressed in young tissues, e.g., hypocotyls (Maeshima, 2000). This is understandable, taking into account that the substrate for V-PPase, inorganic pyrophosphate, is a byproduct of the synthesis of RNAs, proteins, and cellulose. Another situation, when the PPase activity increases in order to compensate decreased activity of H^+-ATPase, takes place under cold stress and anoxia (Rea & Poole, 1993). It has been proposed that PPase might operate as an active K^+/H^+ symporter (1.3 H^+:1.7 K^+:1 PP_i), thus promoting K^+ accumulation into the vacuole against the electrochemical gradient for K^+ (Davies, 1997). However, experiments with purified enzyme reconstituted into liposomes did not show PPase -dependent K^+ accumulation, albeit these data did not abolish the stimulatory effect of K^+ on PPase (Sato et al., 1994). However, later on, a high K_m value for the K^+ stimulation of the PPase activity was proved to be an experimental artifact, due to the competitive inhibition by organic buffers. The true K_m, about a few millimoles defined for K^+, was well below the physiological range for this ion, which makes the regulation of PPase activity by K^+ variation unlikely (Gordon-Weeks et al., 1997). Nevertheless, there are some indications that under salt stress V-PPase is inhibited due to competition between K^+ and Na^+ for the regulatory site (Blumwald et al., 2000). V-PPase requires cytosolic Mg^{2+} in a submillimolar range as a co-factor and is inhibited by Ca^{2+}, either as a free ion, or as a substitute for Mg^{2+} in its complex with pyrophosphate (Maeshima, 2000). In *Arabidopsis* and other organisms, another class of H^+-translocating PPases was found recently, whose activity was completely independent of K^+ (Drozdowicz & Rea, 2001). This (type II) PPase may reside, however, in a membrane other than the tonoplast (Maeshima, 2001).

CREATION OF TRANSTONOPLAST GRADIENTS FOR IONS OTHER THAN H^+

Calcium. The tonoplast contains two transport pathways for active Ca^{2+} accumulation into the vacuole: Ca^{2+}/H^+ (with H^+ to Ca^{2+} stoichiometry $\geq$3) antiporters (Blumwald & Gelli, 1997; Sanders et al., 1999) and plasma membrane-type (P-type) primary Ca^{2+}-pumps (Malmstrom et al., 1997). Seven different genes encoding antiporters belonging to the CAX

family (former nomenclature: CAlcium/ H^+ eXchangers, now CAtion/H^+ eXchangers) and six isoforms for Ca^{2+} pumps belonging to the ACA-family have been identified in the genome of *Arabidopsis thaliana* (Maeshima, 2001; Mäser et al., 2001). The tonoplast Ca^{2+}-pump has a higher affinity for Ca^{2+} (K_m=0.2-1.0 µM) but a lower turnover rate in comparison with the dominant Ca^{2+}/H^+ antiporter CAX1 (K_m=10-15 µM). It was speculated that they operate in a different range of free cytosolic Ca^{2+} concentrations, with the antiporter rapidly lowering excessive Ca^{2+} to the operation range of the Ca^{2+}-pump (Maeshima, 2001). However, a recent study by Geisler and co-workers (2000) revealed that the tonoplast Ca^{2+}-pump *ACA4* is located in small vacuoles rather than in the membrane of the large central vacuole. These data imply a functional difference of these compartments, in particular, a distinct role of the small vacuoles in the formation of Ca^{2+} oscillating patterns (Harper, 2001).

The particular importance of Ca^{2+}/H^+ antiporter was strengthened by experiments on transgenic tobacco plants expressing *Arabidopsis* Ca^{2+}/H^+ antiporter CAX1 (Hirschi, 1999). It appears that the transporter was regulated improperly in the transgenic system (over-expressed), thus decreasing availability of Ca^{2+} in any other compartment but in the vacuole. This resulted in plants with necrotic and chlorotic lesions, dead terminal buds, and undersized roots. And *vice versa*, plants with an underexpressed CAX1 gene were more sensitive to high Ca^{2+} levels (Hirschi, 2001). The closely related Ca^{2+}/H^+ antiporter CAX2 has a broader substrate selectivity (Mn^{2+} and Cd^{2+}) and a higher transport capacity; its expression in tobacco can increase the tolerance to Mn^{2+} (Hirschi et al., 2000).

Magnesium. The activity of the Mg^{2+}/H^+ antiporter has been detected in the lutoid membrane of *Hevea brasiliensis* and tonoplast vesicles from Zea mays roots (Amalou et al., 1992; Pfeifer & Hager, 1993). The antiporter can also transport Zn^{2+} and Cd^{2+} but not Ca^{2+}. Thus, Mg^{2+} appears to be taken up separately from the Ca^{2+}/H^+ antiport pathway, as it was possible to isolate Mg^{2+}/H^+ antiport from Ca^{2+}/H^+ one during a solubilization/ reconstitution procedure. The antiporter has been reported to be electroneutral, i.e., exchanging 1 Mg^{2+} for $2H^+$ (Amalou et al., 1992). Recently, the *Arabidopsis* Mg^{2+}/H^+ exchanger AtMHX (closely related to the CAX-antiporter family and to Na^+/Ca^{2+} exchangers from animal cells) has been cloned and expressed in tobacco (Shaul et al., 1999). This study established that AtMHX is localized in the vacuolar membrane (likely in the membrane of central vacuole due to the co-localization with the (γ-TIP isoform of aquaporin) but not in the plasmalemma or in the endoplasmic reticulum. Furthermore, it is expressed mainly in roots and shoots and to a lesser extent in leaves. Surprisingly, the exchanger was electrogenic; in the presence of a divalent cation (2 mM Mg or 0.2 mM Zn^{2+} or Fe^{2+}) at the cytosolic side, and while the opposite side was acidified, it carried outward currents at tonoplast physiological potentials. Whereas Zn^{2+} or Fe^{2+} have ionic radii similar to Mg^{2+}, the divalent ions of different size (Co^{2+}, Ni^{2+} , Cu^2, and, especially important, Ca^{2+}) alone were inefficient as current inducers, though cytosolic but not vacuolar Ca^{2+} stimulated antiporter-mediated Mg^{2+} uptake. Ectopic overexpression of AtMHX although not changing the total Mg^{2+} or Zn^{2+} content, did result in the increased sensitivity of transgenic plants to high levels of Mg^{2+} or Zn^{2+} in the growth medium (Shaul et al., 1999).

Sodium. The ectopic expression of the *Arabidopsis vacuolar* Na^+/H^+ antiporter AtNHX1 dramatically increased the salt tolerance of these plants (Apse et al., 1999).

Whereas control plants were stunted and chlorotic when watered with a 200 mM NaCl solution, transgenic plants were unaffected, and this tolerance was paralleled by higher activity of the vacuolar Na^+/H^+ antiport. Constitutive vacuolar Na^+/H^+ antiport activity was recorded both in salt-tolerant and salt-sensitive plants, though galophyte plants commonly displayed an increase of the Na^+/H^+ antiport. This may be due to the addition of new antiporter molecules and/or, in some cases, due to the up-regulation of the existing antiporters (Blumwald et al., 2000). Bearing in mind that Na^+ is not an important nutrient in plant cells, and in contrast to animal cells, plant antiporters use electrochemical gradients for H^+ instead of those for Na^+, it was surprising to find about 40 genes encoding homologues of Na^+/H^+ antiporters in *Arabidopsis* (Mäser et al., 2001). One might suspect some novel functions assigned to these tentative Na^+ transporters in plant cells.

Potassium. Although K^+ is a major plant nutrient, the exact mechanisms of the creation of transtonoplast gradients for this ion are still uncertain. A systematic microelectrode study of vacuolar and cytosolic K^+ activity in parallel with transtonoplast electric potential measurements on barley root cells grown in different external K^+ concentrations did show a substantial change in the magnitude and direction of the transtonoplast concentration gradient for K^+, but no significant variation of the electric potential, as a function of K^+ status, was observed (Walker et al., 1996). A K^+-driving force as large as 20-27 mV from vacuole to cytosol at K^+ replete conditions, and from cytosol to vacuole at severe K^+ deficiency, was revealed. The authors concluded that at K^+ replete conditions, K^+ needed to be accumulated into the vacuole actively, via an unresolved mechanism, either by PPase or by H^+/K^+ antiport. Curiously, at K^+ starvation the situation was reversed, and K^+ release into the cytosol turned to be active, condition that could be theoretically achieved by a K^+/H^+ symport (Fernando et al., 1992; Walker et al., 1996). Neither of these hypothetical mechanisms of active K^+ transport has been established to date. However, analysis of the *Arabidopsis* genome revealed six putative K^+/H^+ antiporter homologues (KEA1 through KEA6) belonging to the cation/proton antiporter family 2, CPA2 (Mäser et al., 2001). Some of these antiporters could in principle sequester K^+ into in an acidic compartment, e.g., into the vacuolar lumen. Elucidation of the subcellular localization of KEAs may provide an initial test for this hypothesis.

Anions. Information on the mechanism of transport of anions across the tonoplast is scarce. It is well known that the tonoplast is highly permeable to Cl^- and NO_3^-, and in CAM plants also to malate (Martinoia et al., 1986, Cheffings et al., 1997). The membrane potential difference across the membrane of vacuolar vesicles is dissipated by inorganic anions with a relative potency: $SCN^- > NO_3^- = Cl^- > SO_4^{2-} = HPO_4^{2-}$ (Kästner & Sze, 1987). However, this technique reveals the integral permeability of the tonoplast, without separation between different conductance pathways. Uptake of Cl^-, SO_4^{2-}, and HPO_4^{2-} displayed a saturable kinetics with respect to the substrate concentration and is characterized by a K_m value in the range of few millimoles (Martinoia et al., 2000). Under salt stress, the accumulation of Cl^- and loss of NO_3^- was observed in the epidermis of barley leaves (Leigh, 1997), and under increased light intensity NO_3^- is accumulated in lettuce vacuoles in exchange to other anions (Blom-Zandstra & Lampe, 1985). CAM plants, in contrast to other plants, display a higher tonoplast permeability for malate as compared to Cl^- (Cheffings et al., 1997). These observations corroborate the view that anions are not

transported by unique mechanism and that there might be separate transport routes for Cl^-, NO_3^- and malate across the tonoplast. Accumulation of Cl^- and NO_3^- in the vacuole does not always meet predictions for passive diffusion across the tonoplast, and active (H^+-coupled) mechanisms of anion accumulation in the vacuole might be invoked, depending on the developmental and nutritional status (Zhen & Leigh, 1990; Blumwald & Gelli, 1997).

PASSIVE PATHWAYS FOR ION EXCHANGE ACROSS THE VACUOLAR MEMBRANE: CHANNELS

Ion channels recording. The study of ion currents in biological membranes was revolutionized thanks to the invention of the patch-clamp technique (Hamill et al., 1981). This method is based on the formation of a high resistance (GOhm range) contact between a glass electrode with a tip opening of a few microns and a bare lipid bilayer (cell-attached configuration). The contact is also mechanically stable, so it is possible to isolate the membrane patch included into the pipette (inside-out configuration), which normally contains few ion channel copies. Furthermore, it is possible to study them under fixed voltage conditions and controlled solution content at both membrane sides, in the patch pipette and in the bath. An additional trick is to destroy the membrane under the pipette in the cell-attached configuration by a strong suction and/or voltage pulse, while preserving, however, the GOhm seal. Thus, a low-resistance (few MOhm) access to the cell's or organelle's interior can be achieved, allowing for the detection of ion currents in whole cell (or whole vacuole) configuration. Finally, the excision of the pipette will lead to the excised membrane patch or small vesicle with the right side oriented membrane (outside-out configuration). The patch-clamp registration scheme is a variation of a voltage-clamp method with only two electrodes in use: one is the reference electrode and the other is utilized both for fixing the voltage and measuring the compensatory current which is equal in absolute value to the current passing across the patch membrane. Employing this technique, currents down to 10^{-13} A (~10^5 elementary charges per second) can be easily detected as compared to the current range of 10^{-12}-10^{-11} A (1-10 pA) typical for single ion channels. However, the resistance of the membrane under study must be significantly higher than the patch-electrode resistance, otherwise voltage-clamp conditions will not be met. The typical MOhm resistance of the patch-pipette and the operational voltage range (~0.1 V) results in an upper current detection limit of a few tens of nA (10^{-8} A). This condition is normally fulfilled for small membrane patches but not always for the whole cell (or organelle) configuration. In particular, this applies to large plant vacuoles (several tens of microns in diameter), where major membrane ionic currents may reach or even exceed the aforementioned upper limit. Therefore, it is possible using a patch-pipette to separate smaller membrane vesicles from the large vacuole, whose higher membrane resistance allows a correct voltage-clamp detection.

SV and FV channels. Due to its large size and clean membrane surface, the vacuole is a relatively simple object for patch-clamp studies. The first ion channel to be characterized in the vacuolar membrane was called the SV (Slow Vacuolar) channel of red beet vacuoles (Hedrich et al., 1986). The SV current was both voltage- (activated by cytosol positive voltage) and time-dependent (required hundreds of milliseconds to

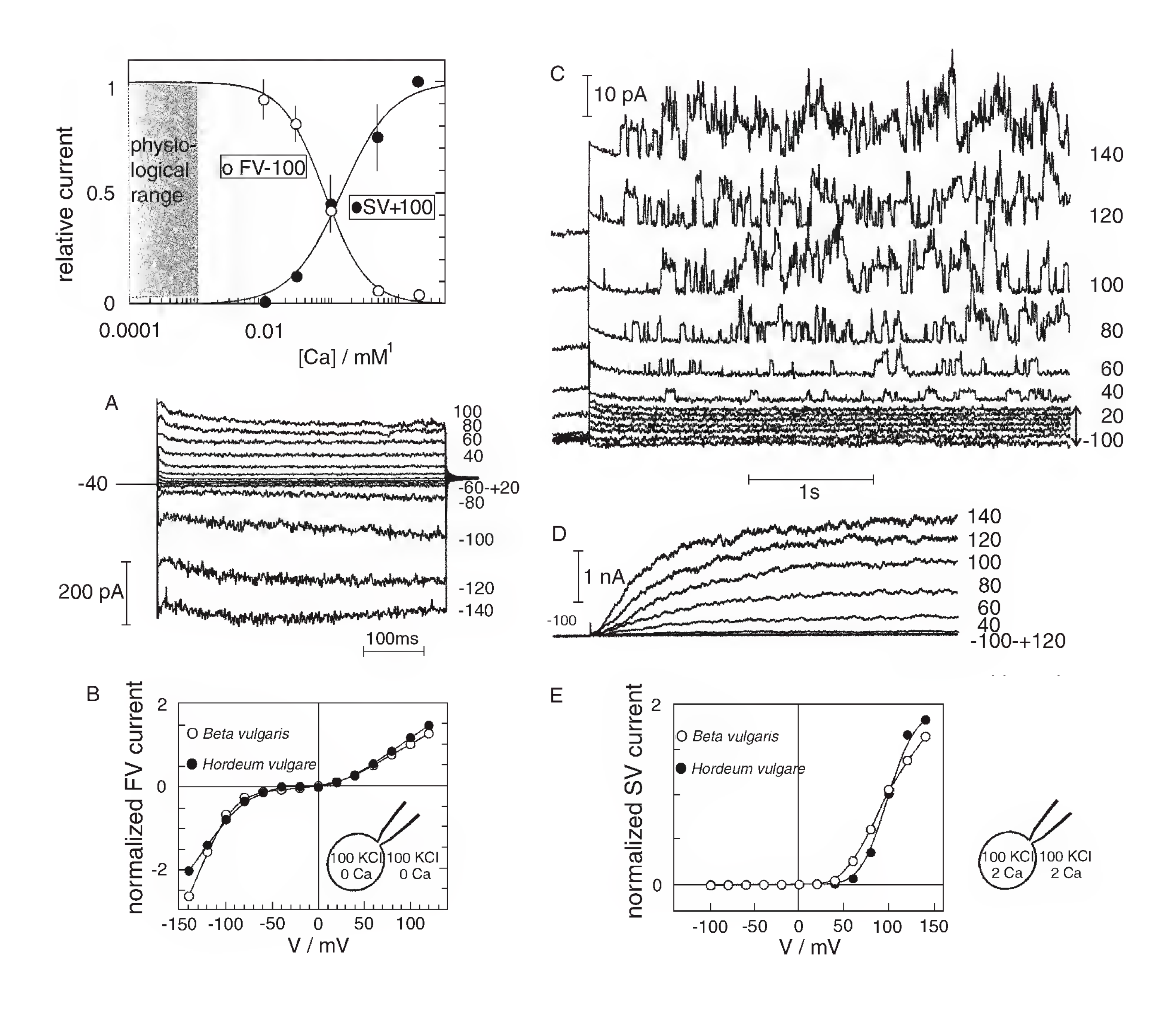


Fig. 3. Two major ionic currents of higher plant vacuoles. All records shown were obtained in symmetrical 100 mM KCl, pH 7.5. Original data are from red beet (*Beta vulgaris*) vacuolar vesicles, diameter $d \leq 5$ µm. A. At low (nM) free Ca^{2+} on both membrane sides, the membrane conductance is dominated by the FV (Fast Vacuolar) current, which is activated instantaneously by the application of positive and negative voltage steps from a holding potential of –40 mV, where the FV channel activity is minimal. B. Similar voltage dependence of the normalized FV current in vacuoles of red beet taproots (*Beta*) and of barley mesophyll (*Hordeum*). C. High (2 mM) cytosolic Ca^{2+} abolishes the FV current and activates the SV (Slow Vacuolar) channels. Free vacuolar Ca^{2+} was also increased to 2 mM, so under these conditions only positive membrane voltage steps ($\geq$40 mV) evoke SV channels activity in a tiny (d<1 µm) tonoplast patch. D. The record from a larger vacuolar vesicle under conditions identical to C reveals delayed activation of macroscopic SV current by positive voltage steps. E. The SV current shows saturable activation by membrane voltage, with a midpoint about +90 mV both in red beet and in barley mesophyll vacuoles. Insert. Shows opposite dependence of FV (at –100 mV) and SV (at +100 mV) currents in red beet vacuoles on cytosolic free Ca^{2+}, with a midpoint for the inhibition of the FV and the activation of the SV at Ca^{2+} ~100 µM.

Table 1. Characteristics of cation channels in higher plant vacuoles.

Parameter	FV channel	SV channel
Conductance		
K^+	21 pS (100 mM) K_m=30 mM (*Hordeum*[a]); 7 pS (200 mM) (*Vicia*[b]); 21 pS (100 mM) (*Beta*[C])	K_m=64 mM; G_{max}=385 pS (*Beta*[h]) K_m~550 mM; G_{max}~2000 pS (*Vicia*[i])
Na^+	15 pS (100 mM) (*Hordeum*[d])	K_m=103 mM; G_{max}=300 pS (*Beta*[h])
Mg^{2+} & Ca^{2+}	unmeasurably (<5% of K^+) low (*Hordeum*[a,e])	K_m=0.04 & 0.08 mM; G_{max}=18 & 13 pS (*Beta*[h]) (Ca^{2+}) K_m~6.5 mM; G_{max}~35 pS (*Vicia*[i])
Selectivity	NH_4^+>K^+~Rb^+~Cs^+>Na^+>Li^+(*Hordeum*[d]) K^+>>Cl^- (*Hordeum*[a], *Vicia*[b])	Na^+>K^+>Rb^+>Cs^+ (*Allium cepa* guard cell[j]) K>>Cl^-; Ba^{2+}>Ca^{2+}>Mg^{2+} (*Beta*[h,k], *Vicia*[i])
Blockers		
Physiological	vacuolar Mg^{2+} & Ca^{2+} (V-dependent) $K_d(0)$~40 μM & 200 μM[e]	vacuolar and cytosolic Mg^{2+} & Ca^{2+} (V-dependent) $K_d(0)$~1-3 mM (*Beta*[k]) Vacuolar / cytosolic polyamines: Spm^{4+}> Spd^{3+}>Put^{2+} (V-dependent), $K_d(0)$: 60/30; 400; 8,000/2,000 μM (*Beta*[l])
Non-physiological	not determined	cytosolic application:DIDS or SITC (1 μM); Zn^{2+} (5 μM); A-9-C (100 μM); charibdotoxin (20 nM); tubocurarine (60 μM); quinacrine (15 μM); ruthenium red (<0.1μM; 36 μM); TEA^+ (10 mM); $Tris^+$ (60 mM) ([m-p])
Regulation		
Voltage (V)	activation by positive and negative V, V_{min} 20-40 mV negative to reversal V Gating charge(z) ~1.2 elem. charges[a,b,c]	activation by positive V, midpoint is a function of cytosolic Ca^{2+} and Mg^{2+} and of vacuolar Ca^{2+}. At symmetrical 1-2 mM Ca^{2+} V_m~90 mV; z=+1.4, +1.7 (*Beta*[k], *Hordeum*[q]) V_m ~60 mV z=+3.9 (*Vicia*[r])
pH	5-fold inhibition by pH change from 7.4 to 6.4[b]	(-) modulation by cyt. and vac. protons; pK= 6.9 and 5.0 (*Beta*[r]); vac. pH regulation insignificant at vac. Ca^{2+}>50 μM (*Hordeum*[q])
Ca^{2+} & Mg^{2+}	Cyt Ca^{2+}- inhibition, K_i=: 6 mM (*Hordeum*[a]); ~0.1 μM (*Vicia*[f]); 80 μM (*Beta*[c]); cyt. Mg^{2+}-stabilization of closed state; K_i (0)=10 μM[e]	High cytosolic Ca^{2+} is absolute requisite for activation; K_d~100 μM at +100 mV (*Beta*[k]; *Hordeum*[q]; *Vicia*[s]). Rise in vacuolar Ca^{2+} opposes activation by cyt. Ca^{2+}; cyt. Mg^{2+} is backing the effect of cyt. Ca^{2+}, vac. Mg^{2+} practically has no effect ([k,q,s])
Other	Cytosolic polyamines- inhibition: Spm^{4+}>Spd^{3+}>>Put^{2+}, K_i: 3-10; 75-185; ~ 5,000 μM (*Beta*[c]; *Hordeum*[g])	(+) modulation by cytosolic Cl^- ([t]), cal-modulin ([s,u]), reducing agents ([v]), (+/-) modulation by phosphorylation status ([w,x]), (-) modulation by 14-3-3 proteins ([y])

seconds for its activation). Subsequently, SV channel activity was recorded in a wide variety of tissues, in all flowering plants, lower terrestrial plants such as ferns and liverworts, and even in a sea grass *Posidonia oceanica* (Hedrich et al., 1988; Schulz-Lessdorf & Hedrich, 1995; Carpaneto et al., 1997; Trebacz & Schönknecht, 2000). SV channel activity has also been found in specialized vacuoles such as the protein storage vacuole of the cereal aleuron tissue (Bethke & Jones, 1994). Another ubiquitous tonoplast current, which is differed from the SV current by its instantaneous activation, is mediated by the so called FV (Fast Vacuolar) channel (Hedrich & Neher, 1987). It differs from the SV channel also by its bimodal voltage dependence (activation at large positive and negative potentials) and the opposite Ca^{2+} dependence (Fig. 3). Single channels responsible for macroscopic SV and FV currents have been identified, and the SV channel has a ~10-fold higher conductance in comparison with the FV channel at identical ion conditions (Table 1). Based on single channel conductance values, at least two isoforms of the FV channel as well as of SV channel, likely exist in different tissues (Schulz-Lessdorf & Hedrich, 1995; Table 1 of this paper). In particular, the SV channel from guard cells is characterized by the highest unitary conductance along with a lower affinity for permeant ions than SV channels of other origin.

The FV channel conducts monovalent cations with a small preference $NH_4^+ > K^+ \sim Rb^+ \sim Cs^+ > Na^+ > Li^+$ (Brüggemann et al., 1999a). It was shown that the FV channel is highly selective for K^+ over Cl^-, and that it is inhibited by micromolar Mg^{2+} or Ca^{2+} from either membrane side (Tikhonova et al., 1997; Allen et al., 1998a; Brüggemann et al., 1999b). The ionic selectivity of the SV channel was a matter of hot debate. Whereas Hedrich and co-workers (Hedrich et al., 1986; Hedrich & Kurkdjian, 1988; Schulz-Lessdorf & Hedrich, 1995) claimed that the SV channel conducts both cations and anions, reports from other groups (Ward & Schroeder, 1994; Allen & Sanders, 1996; Pottosin et al., 1997; Allen et al., 1998b) supported the view that the anion permeability of the SV channel is negligible. Finally, it has been shown that Cl^- permeability of the SV channel is at most 1% that of K^+, and that the SV channel conducts the following physiologically abundant cations at high rate: Na^+, K^+, Mg^{2+}, and Ca^{2+} (Pottosin et al., 2001). Besides the aforementioned cation species, the SV channel also conducts Ba^{2+} (Pantoja et al., 1992a) and all alkali cations with a small preference (Amodeo et al., 1994). Both FV and SV

Table 1 footnote:
(a) Tikhonova et al., 1997; (b) Allen et al., 1998a; (c) Dobrovinskaya et al., 1999ª; (d) Brüggemann et al., 1999ª ; (e) Brüggemann et al., 1999ᵇ; (f) Allen & Sanders (1996); (g) Brüggemann et al., 1998; (h) Pottosin et al., 2001; (i) Allen et al., 1998b; (j) Amodeo et al., 1994; (k) Pottosin et al., 2000b; (l) Dobrovinskaya et al., 1999a; (m) Hedrich & Kurkdjian, 1988; (n) Weiser & Bentrup, 1993; (o) Dobrovinskaya et al., 1999b; (p) Pottosin et al., 1999; (q) Pottosin et al., 1997; (r) Schulz-Lessdorf & Hedrich, 1995; (s) Pei et al, 1999; (t) Pantoja et al. 1992a; (u) Bethke & Jones, 1994; (v) Carpaneto et al., 1999; (w) Allen & Sanders, 1995, (x) Bethke & Jones, 1997; (y) van den Wijngaard et al. (2001). *Hordeum*- barley (*Hordeum vulgare*) mesophyll vacuoles; *Vicia* – broad bean (*Vicia faba*) guard cell vacuoles; *Beta*- sugar beet (*Beta vulgaris*) taproot vacuoles. Positive and negative modulation indicated by (+) and (-), respectively. If not specified concentrations of effectors given are those to produce half-effect at 0 mV. Abbreviations: V- voltage, cyt.- cytosolic, vac.- vacuolar, V_{min} – voltage value for minimal activity of FV channel. Direct effect at the pore (block) is separated from inhibition with unknown mechanism of action, which in defined cases can be identified with down-regulation.

channels are therefore strictly selective for cations over anions, but in contrast to the SV channel, the FV channel does not show a measurable permeability for divalent cations.

VK channels. In the guard cell vacuolar membrane, a separate, highly K^+ selective channel with a unitary conductance of 70 pS in symmetrical 100 mM KCl was characterized (Ward & Schroeder, 1994). The selectivity sequence of this channel, termed VK (for Vacuolar K^+), $K^+>Rb^+>NH_4^+>>Li^+$, Na^+ or Cs^+ , clearly differed it from the relatively non-selective FV and SV channels. The activity of VK was independent of membrane voltage and required cytosolic Ca^{2+} in the low micromolar range (Ward & Schroeder, 1994; Allen et al., 1998a). We (Pottosin et al., in press) have identified in the taproot of the halophyte plant *Beta vulgaris* the 41 pS K^+-selective channel with a negligible Na^+ permeability. Besides lower unitary conductance, the channel has quite similar functional characteristics to those of the VK channel of guard cells (Ward & Schroeder, 1994), and it was identical to the *B. vulgaris* vacuolar 30-40 pS cation channel, loosely defined as "FV-like" in previous studies (Hedrich & Neher, 1987; Gambale et al., 1996). So far, the VK channel is the only strictly K^+-selective channel to be identified in the vacuolar membrane. Such a property might be important for plants under salt stress. To cope both with hypertonic stress and Na^+ toxicity, salt-tolerant plants import Na^+, as a cheap osmoticum, into the vacuole (Blumwald et al., 2000). This is achieved by an enhanced activity of vacuolar Na^+/H^+ antiporter energized by H^+-pump (mainly by V-ATPase). However, the overall system turns out to be electrogenic, i.e., a net positive charge is transported into the vacuole. This needs to be balanced, otherwise the long-term transport of Na^+ into the vacuole cannot be maintained. Balancing the charge movement by a cation leak via non-selective FV and SV channels does not make sense, as these channels are highly permeable for Na^+, and futile Na^+ cycling across the tonoplast will arise. On the contrary, a K^+ leak through a K^+-selective (VK?) channel can help solve the problem[2]. Recently, one potassium channel homologue (KCO1) has been localized in the vacuolar membrane of *Arabidopsis* (Schönknecht et al., 2002). Future studies will hopefully elucidate its relation to known functional vacuolar cation channels, as well as its physiological role.

VVCa. Another Ca^{2+}-permeable channel, with voltage and Ca^{2+}-dependence opposite to those of the SV channel, was reported by studies of vacuoles from red beet and Vicia guard cells (Johannes et al., 1992; Allen & Sanders, 1994b; Johannes & Sanders, 1995). This channel was termed VVCa in subsequent review papers (Allen & Sanders, 1997; Sanders et al., 1999). A quantitative comparison of the conductance, selectivity, and absolute values of parameters of Ca^{2+} and voltage-gating of the VVCa and SV channels from the same preparation revealed their complete equality (Pottosin et al., 2001). This result was very striking, bearing in mind that SV channels from storage and guard cell vacuoles differ substantially in some characteristics (Table 1). Therefore, we (Pottosin et al., 2001) have concluded that the VVCa channel is actually the same as the SV channel, recorded on the inverted tonoplast patches. Such a technical error could

[2] The algebraic sum H^+-pump plus Na^+/H^+ antiporter plus K^+ channel is equivalent to Na^+ / K^+-ATPase of animal cells, a constitutively active enzyme consuming a major portion of cellular ATP to maintain appropriate Na^+ / K^+ balance under conditions of a permanent "salt stress", i.e., high (>100 mM) Na^+ in the extracellular medium.

occur while working with excised patches, but not with whole vacuoles. Consequently, the VVCa-mediated current has never been demonstrated at the whole vacuole level, albeit some inwardly rectifying current carried by Ca^{2+}, with characteristics distinct from VVCa, was detected in red beet vacuoles (Gelli & Blumwald, 1993). However, the existence of Ca^{2+} permeable channels open at physiological (cytosolic-side negative) potentials and resting cytosolic Ca^{2+} conditions is highly unlikely on physiological grounds, because a continuous Ca^{2+} release to the cytosol from the vacuolar store will be fatal to the cell.

Tonoplast ligand-gated Ca^{2+} release channels? In animal cells two principle families of intracellular ligand-gated Ca^{2+} release channels are recognized. They are channels activated by inositol 1,4,5-triphosphate (IP_3), a product of phospholipase C activity, and ryanodine receptor (RyR) channels, activated by cyclic adenosinephospate ribose (cADPR), a NAD^+ metabolite, respectively (Ehrlich et al., 1994; Guse, 1999). Experiments with caged IP_3 demonstrated the liberation of Ca^{2+} from internal stores in *Vicia faba* L. guard cells (Blatt et al., 1990). IP_3-induced Ca^{2+} release was detected *in vitro*, using Ca^{2+}-selective electrodes and fluorescence Ca^{2+} measurements on intact vacuoles and on tonoplast vesicles from *Chenopodium album* root cells (Lommel & Felle, 1997). *In vivo* Ca^{2+} measurements in the vacuolar microdomain of *Arabidopsis* seedlings have shown a significant contribution of IP_3-induced Ca^{2+} release from the vacuole during hyperosmotic (drought) stress, whereas, touch, cold and oxidative stress caused a reduced Ca^{2+} peak in this domain as compared to the Ca^{2+} rise in the cytosol (Knight et al., 1997). Therefore, the search for ligand-gated intracellular Ca^{2+}-permeable channels in the tonoplast of plant cells has been initiated. Alexandre and co-workers (1990) working on red beet vacuoles reported large (~500 pA at -100 mV) inwardly rectifying currents, mediated by 30 pS Ca^{2+}-permeable channels, specifically activated by submicromolar IP_3 and not by other phosphoinositides. Unlike animal IP_3-gated channels, these putative channels were not inhibited by high (1 mM) cytosolic Ca^{2+}. However, these results have not been reproduced by other groups (Chasan & Schroeder, 1992; Gelli & Blumwald, 1993). It has been claimed then (Allen & Sanders, 1994a) that the origin of the discrepancy was that Alexandre and co-workers (1990) used a hypertonic treatment of vacuoles, which favors the IP_3-induced current. Using a hypertonic treatment, Allen and Sanders (1994a) repeatedly recorded an increase in the whole vacuole current in the presence of 1 µM IP_3. However, this was in on the background of a large, unspecific leak, and comparable with the current supposedly induced by IP_3. No unambiguous single IP_3-gated channels could be demonstrated in this work. The situation has become even more complicated in the light of recent studies on purified microsomal fractions obtained from cauliflower and *Chenopodium rubrum* L. leaves (Muir & Sanders, 1997; Martinec et al., 2000). The first study, based on the IP_3-induced Ca^{2+} release assay and cross-reaction with antibodies raised against mammalian type 1 IP_3 receptor, found IP_3 receptors mainly in fractions enriched with plasma membranes in close association with endoplasmic reticulum (ER), whereas the second study localized IP_3-binding sites exclusively in ER fractions, but not in vacuolar ones.

In guard cells, the cADPR-linked signal transduction pathway plays an important role in ABA-induced response. ABA causes an increase of the intracellular cADPR level, and ABA-induced gene expression can be stimulated by microinjection of cADPR, being prevented by cADPR antagonist. Although in the absence of ABA both high cytosolic Ca^{2+} and IP_3 microinjection could mimic the effect, the natural ABA-induced response was

insensitive to heparin, an IP_3 receptor channel blocker (Wu et al., 1998). In *Vicia faba* guard cells, Ca^{2+}-induced Ca^{2+} release (CICR) caused by ABA was stimulated rather than inhibited by the IP_3 channel blockers neomycin sulfate and heparin, whereas high (10 µM) ryanodine concentrations antagonized the response (Grabov & Blatt, 1999). Because of an unspecific stimulation of the ryanodine receptor channel by polyanions, e.g., heparin, and its inhibition by high ($\geq$10 µM) ryanodine concentrations (Ehrlich et al., 1994), it may be concluded that ABA-induced CICR is mediated by the ryanodine/cADPR receptor channel. The direct injection of cADPR in guard cells of *Commelina communis* results in a Ca^{2+} rise, more commonly in a sustained form rather than in the wave or transient fashion, typical for IP_3 response observed in other cells, and ABA-induced Ca^{2+} release could be antagonized by 8-NH_2-cADPR and the byproduct of APP-ribosyl cyclase nicotinamide (Leckie et al., 1998a). Thus, cADPR-mediated release of Ca^{2+} (but not IP_3-mediated release) from intracellular stores is involved in the ABA-induced signal transduction pathway. However, the cellular localization of cADPR/ryanodine receptor could not be specified in these studies.

Allen and coworkers (1995) found that IP_3 and cADPR could induce Ca^{2+} release from red beet microsomes in an additive manner, which implies that in their microsomal preparation there were separate vesicle fractions containing different Ca^{2+} release pathways. Searching for cADPR receptor channels, the patch-clamp technique was applied to intact vacuoles and a mildly inward rectifying ionic current, seen as a small increment on the background of an unspecific leak, was attributed to the cADPR action (K_m ~ 25 nM). This current showed low sensitivity to ruthenium red (30-100 µM) and to ryanodine (10 µM), a blocker and a modulator of the animal RyR channel, respectively[3]. In guard cell vacuoles, attempts have been also made to identify the cADPR-activated current by means of the patch-clamp technique (Leckie et al., 1998a). In contrast to previous observations in red beet vacuoles, the "cADPR-induced" currents were inhibited by cytosolic Ca^{2+} with K_m ~100 nM. This value corresponds to the Ca^{2+} sensitivity of the FV current in this preparation (Allen & Sanders, 1996). Taken together with the FV-like current-voltage relationship of the current increment posterior to the cADPR application, it is likely that Leckie et al. (1998a) actually recorded a "run-up" of the FV channel activity (Allen et al., 1998a). Animal RyR channels are large (several hundred pS) conductance ones. They are activated by micromolar (and inhibited by hundred micromolar) cytosolic Ca^{2+} concentrations which underlies their function in CICR (Smith et al., 1985; Ehrlich et al., 1994). In studies on plant cells, no such channels have been detected to date.

Although the existence of IP_3- and cADPR-gated channels in the vacuolar membrane has gained broad acceptance (Muir et al., 1997; Leckie et al., 1998b; Thuleau et al., 1998; Sanders et al., 1999), we think that this notion deserves further proof. Surprisingly, no homologues for animal IP_3 or RyR channels were found in the *Arabidopsis* genome. Does this mean that plant ligand-gated channels have unique sequences? What are, then, their functional properties in comparison with their animal counterparts? Are ligand-gated Ca^{2+} release channels located in the vacuoles (cADPR-activated channel?), in the ER (IP_3-gated

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[3] RyR channels from animal cells are irreversibly modified (gated open) by ~0.1 µM and subsequently blocked by 10 µM of ryanodine (Ehrlich et al., 1994). RyR channel is completely blocked by 1 µM of RR (Smith et al., 1985). For a comparison, the Ca^{2+} -permeable SV channel from the same vacuolar preparation (red beet) is inhibited by submicromolar RR (Pottosin et al., 1999).

channel) or in some other intracellular stores? Answering these questions will bring substantial progress towards our understanding of intracellular Ca^{2+} signaling in plants.

Tonoplast anion channels. Preliminary patch-clamp studies of anion channels in vacuolar membranes of different origin revealed a broad range of unitary conductance values, from 7 pS up to 125 pS (Iwasaki et al., 1992; Plant et al., 1994; Pei et al., 1996). Tentative tonoplast channels appear to conduct a wide spectrum of inorganic (Cl^-, NO_3^-, HPO_4^{2-}) and organic (acetate, oxaloacetate, succinate, fumarate, and malate) anions (Iwasaki et al., 1992; Plant et al., 1994; Cerana et al., 1995). It is not clear how many anion channel types are at work. However, there are several lines of evidence for the involvement of multiple channel types. Plant and co-workers (1994) observed that high vacuolar Cl^- down-regulates inward anion currents. The residual current as compared to the initial one was characterized by a relatively low permeability for Cl^- and malate as compared to acetate and NO_3^-. In the presence of malate, some fraction of the whole vacuole current carried by anions became time-dependent, with a characteristic time in the range of seconds (Pantoja et al., 1992b; Cerana et al., 1995; Pei et al., 1996; Cheffings et al., 1997). Whether malate modifies existing channels or induces novel, slow type channels, remains unclear, although several authors use separate names, VCL and VMAL, to designate vacuolar Cl^- and malate-permeable channels, respectively. Mechanisms of the tonoplast's permeability for Cl^- and malate^{2-} differ, in fact, between C_3 and CAM plants. The tonoplast of CAM plants generally displays a larger passive permeability to malate relative to Cl^-, and the rate of malate transport is correlated with the degree of CAM activity (reviewed by Cheffings et al., 1997).

REGULATION OF THE VACUOLAR ION CHANNELS' FUNCTION

To date only three cation channels (SV, FV and VK) and an anion channel species (VCL of guard cells) are unambiguously identified in the tonoplast and explored to such an extent that allows the elucidation of their regulation by a variety of physiologically relevant factors.

Voltage. Among the aforementioned vacuolar channels, only vacuolar potassium (VK) channels are insensitive to a variation of the voltage difference across the tonoplast (Ward & Schroeder, 1994; Allen et al., 1998a).

The SV channel is an outward rectifier, i.e., it is activated by positive voltage steps (Fig. 3 C-E) favoring monovalent cation influx into the vacuole. The voltage dependence of its opening probability is further shifted by monovalent cations (e.g., K^+), and by Ca^{2+} and Mg^{2+}, whereas K^+ fluxes through the open channel are blocked in a voltage-dependent manner by divalent cations and polyamines (see below).

The unblocked FV channel has a carrier-like, bipolar κ-shape voltage dependence (Fig. 3 A-B), with a minimal activity around the vacuolar resting potential (-40 mV) and increased activity at higher potentials of either direction. The position of the minimum follows a shift of equilibrium potential for permeable cation (K^+), see regulation by permeable ions. Thus, the FV channel, as proposed by Tikhonova and co-workers (1997), might operate as functional K^+ channel, clamping the tonoplast voltage close to E_K+.

Further, monovalent cation fluxes via FV channels are strongly rectified by the vacuolar divalent cations Ca^{2+} and Mg^{2+} (see below).

The common property of anion currents reported by different authors is their inward rectification (Iwasaki et al., 1992; Pantoja et al., 1992b, Plant et al., 1994; Cerana et al., 1995; Pei et al., 1996; Cheffings et al., 1997). This means that tentative anion channels are suited for anion uptake into the vacuole but not for the anion release. Stomatal closure, for instance, requires a sustained anion efflux amounting to ~100 pA ($6x10^8$ ions/s). Such a flux may not be achieved by, so far unique, guard cell anion channel (activated by the calmodulin-like domain protein kinase, CDPK) described by Pei and co-workers (1996). Consequently, it was proposed that guard cell anion channels promote the stomatal opening instead. Similarly, in CAM plants a role for anion channels in the nocturnal malate accumulation was postulated (Cheffings et al., 1997), whereas the mechanism of malate efflux from the vacuole in light remains elusive.

Permeable ions. The voltage difference between the cytosol and the vacuole appears not to change drastically, being between +5 and -20 mV as reported by microelectrode measurements. Therefore, it is not voltage dependence itself but its modulation by physiological factors that may play an important role in the vacuolar channels' gating. In contrast to animal cells, where drastic changes of membrane voltage, known as action potentials, are caused by tiny amounts of ions flowing via membrane channels, plant ion channels can mediate large changes of intracellular ion contents (see for an example "osmotic motors" above). It is unclear whether ion channels sense these changes and/or are regulated by their own substrates, as it is true for carriers, where ion translocation and gating are intrinsically coupled. Variability of the vacuolar ion content might be especially important, as cytosolic ion concentrations generally need to be tightly controlled.

One example of such a mechanism is the regulation of the SV channel by the cytosolic and vacuolar Ca^{2+}, when the open probability of the channel became the function of electrochemical potential difference for Ca^{2+},) μCa^{2+} (Pottosin et al., 1997). Due to the unique role of Ca^{2+} in signaling, this mechanism) will be considered separately below. Apart from the modulation by Ca^{2+}, which was highly specific, SV channel voltage gating is affected by other cations from the vacuolar side. Thus, the increase of vacuolar K^+ (10-400 mM range) in the presence of submillimolar vacuolar Ca^{2+} caused a negative shift of the voltage dependence (Pottosin & Martínez, unpublished). This effect was interpreted as a consequence of a dilution of the local Ca^{2+} concentration due to the screening of the negative surface charge by K^+ and other monovalent ions.

The gating of FV channels senses the variations in the permeable cation species and concentration. Inward (cytosol-directed) currents via FV channels at bi-ionic conditions (KCl inside the vacuole and XCl in the cytosol) are decreased strictly in accordance with the relative permeability of the cation X (Brüggemann et al., 1999a), which was caused by the decrease of the open channel probability (Fig. 4). Variation in the cytosolic K^+ on the other hand equally shifts the reversal potential and the position of the minimum of the FV current voltage dependence in vacuoles of *Hordeum vulgare* mesophyll and *Vicia faba* guard cells (Tikhonova et al. 1997; Allen et al., 1998a). This observation was extended by testing the effects of cytosolic and vacuolar K^+ on the FV channel in *B. vulgaris* taproot vacuoles. Remarkably, the position of the minimum is always set ~50 mV below the

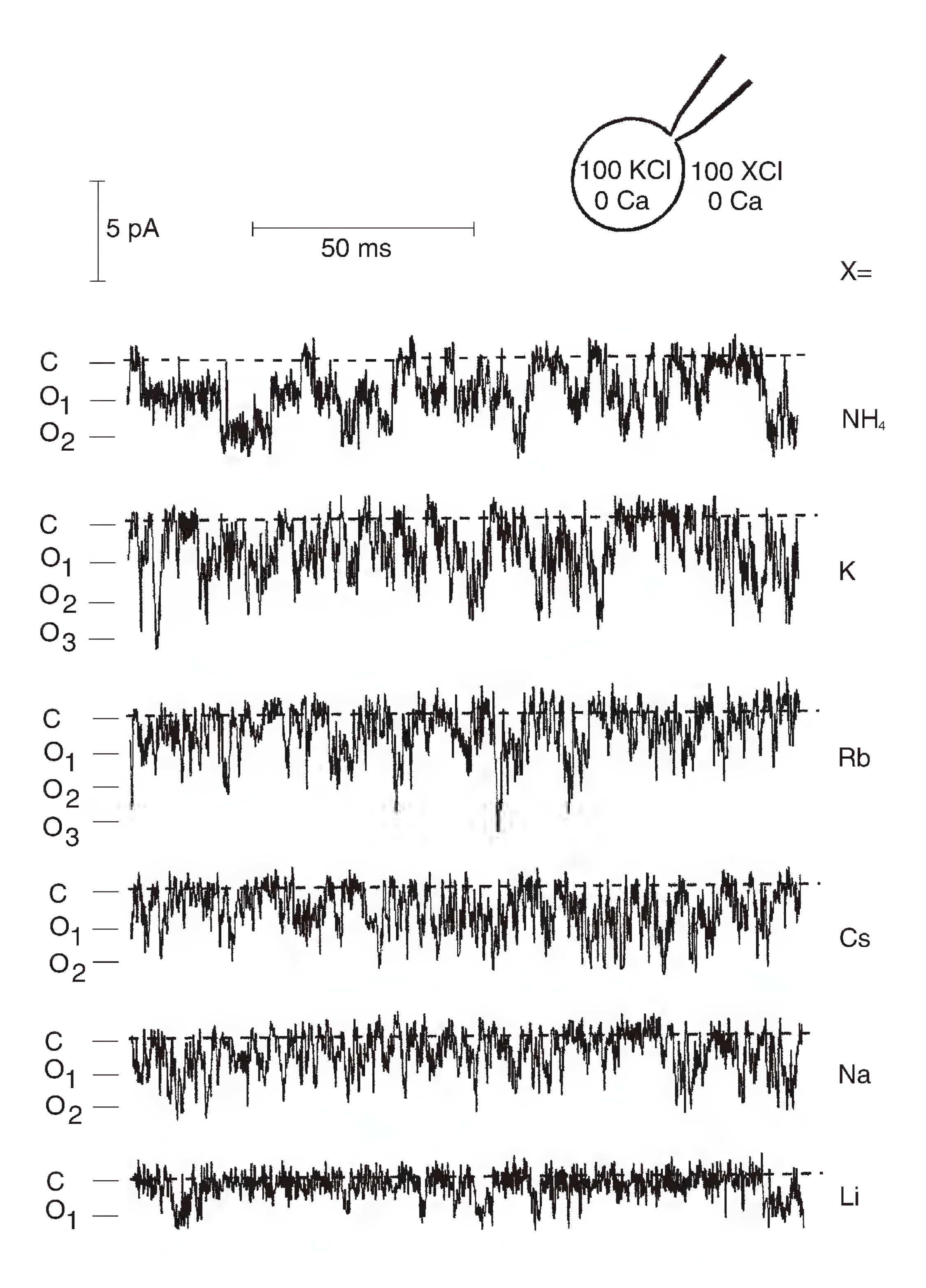


Fig. 4. Gating of the FV channel in barley mesophyll vacuoles is dependent on the permeable cation species. Channel recording at –80 mV from a single cytosolic side-out patch in bi-ionic conditions with 100 mM KCl at the vacuolar side and 100 mM XCl at the cytosolic side, where X is a variable, monovalent cation species. Free Ca^{2+} at both membrane sides is set to the nanomolar level. Indexes at the left hand side correspond to no (C), one, two or three (O_1, O_2, O_3) FV channels open. Ionic currents under these conditions are carried by K^+ in all cases. The sequence from top to bottom corresponds to the relative permeability sequence of the FV channel, and the decrease in relative activity parallels the decrease in relative permeability (Tikhonova, Pottosin & Schönknecht, unpublished data).

equilibrium potential for K^+ (Martínez & Pottosin, unpublished). In the physiologically attainable tonoplast potential range, the FV open probability was mainly dependent on vacuolar K^+: the larger the vacuolar K^+ concentration, the higher the channel activity. Down-regulation of the FV channel by decreased vacuolar K^+ could be important at K^+ starvation, in order to prevent passive K^+ re-uptake into the vacuole when the vacuole to cytosol electrochemical gradient for K^+ is reverted from the cytosol-directed to the vacuole-directed one (Walker et al., 1996). Noteworthy, both vacuolar cation channels (FV and SV) display the valve-like behavior: they are down-regulation by a decrease in vacuolar K^+. Previously, MacRobbie (1998) discovered that the ion channel-mediated loss of the radioactive analogue of K^+ ($^{86}Rb^+$) during ABA-induced stomatal closure is regulated in a feedback manner by the $^{86}Rb^+$ content in the vacuole. She hypothesized that some yet unidentified stretch-activated channels might be involved. Our finding that vacuolar K^+ content affects the open probability of the two principle vacuolar cation channels, FV and SV, might provide a more direct explanation for the observed feedback control.

Anion currents in *Arabidopsis* vacuoles can be modulated by cytosolic malate concentrations, with a threshold for activation shifting more positive at increased malate, but in such a way that always favors the malate influx (Cerana et al., 1995). Increase of the vacuolar Cl^- in the presence of Cl^- or malate at the cytosolic side also increased the voltage threshold; curiously, an opposite effect was observed when nitrate was a sole anion in the cytosolic solution (Plant et al., 1994). Further studies need to be conducted in order to resolve the impact of all these factors. In any case, it is important to learn that, as FV and SV channels, tentative tonoplast anion channels seem to be modulated by permeable ions, which potentially allows a feedback control of the channel function.

Calcium. Cytosolic Ca^{2+} is required for the activation of the VK and anion channels in guard cell vacuoles (the latter via phosphorylation by Ca^{2+} dependent kinase, CDPK) (Ward & Schroeder, 1994; Allen & Sanders, 1996; Pei et al., 1996). Cytosolic Ca^{2+} inhibits FV channels in a voltage-independent manner (Allen & Sanders, 1996; Tikhonova et al., 1997; Dobrovinskaya et al., 1999a). However, only guard cell FV channels were reported to sense cytosolic Ca^{2+} changes in the physiological range (Allen & Sanders, 1996), whereas FV channels of different origin were inhibited by many-fold higher Ca^{2+} activity (Fig. 3, Table 1). For the SV channel, cytosolic Ca^{2+} is a positive modulator of its voltage dependence (Reifarth et al., 1994; Schulz-Lessdorf & Hedrich, 1995; Pottosin et al., 1997), although in the virtual absence of cytosolic Ca^{2+}, voltage-dependent SV currents may be recorded, albeit above the physiological voltage range (Carpaneto et al., 2001).

Due to its activation by a rise in cytosolic Ca^{2+}, Ca^{2+}-permeability and localization in the membrane of an inexhaustible Ca^{2+}-store, the SV channel was considered as a prime candidate for the Ca^{2+}-induced Ca^{2+} release (CICR) in guard cells (Ward & Schroeder, 1994). Indeed, due to a huge cytosol-directed Ca^{2+} gradient (Fig. 1), the SV channel, when open, will mediate Ca^{2+} release from the vacuole. However, it has been found that the SV channel is down-regulated by vacuolar Ca^{2+} (Pottosin et al., 1997). Whereas an increase of cytosolic Ca^{2+} shifted the voltage threshold more negative (Fig. 5A-B), an equivalent increase of vacuolar Ca^{2+} caused the opposite shift. Therefore, at a given Ca^{2+} gradient (defined by equilibrium potential for Ca^{2+}, E_{Ca}) no matter what the absolute Ca^{2+} concentrations at both membrane sides were, the voltage dependence will be the same. Moreover, the voltage activation threshold and E_{Ca} were related linearly with

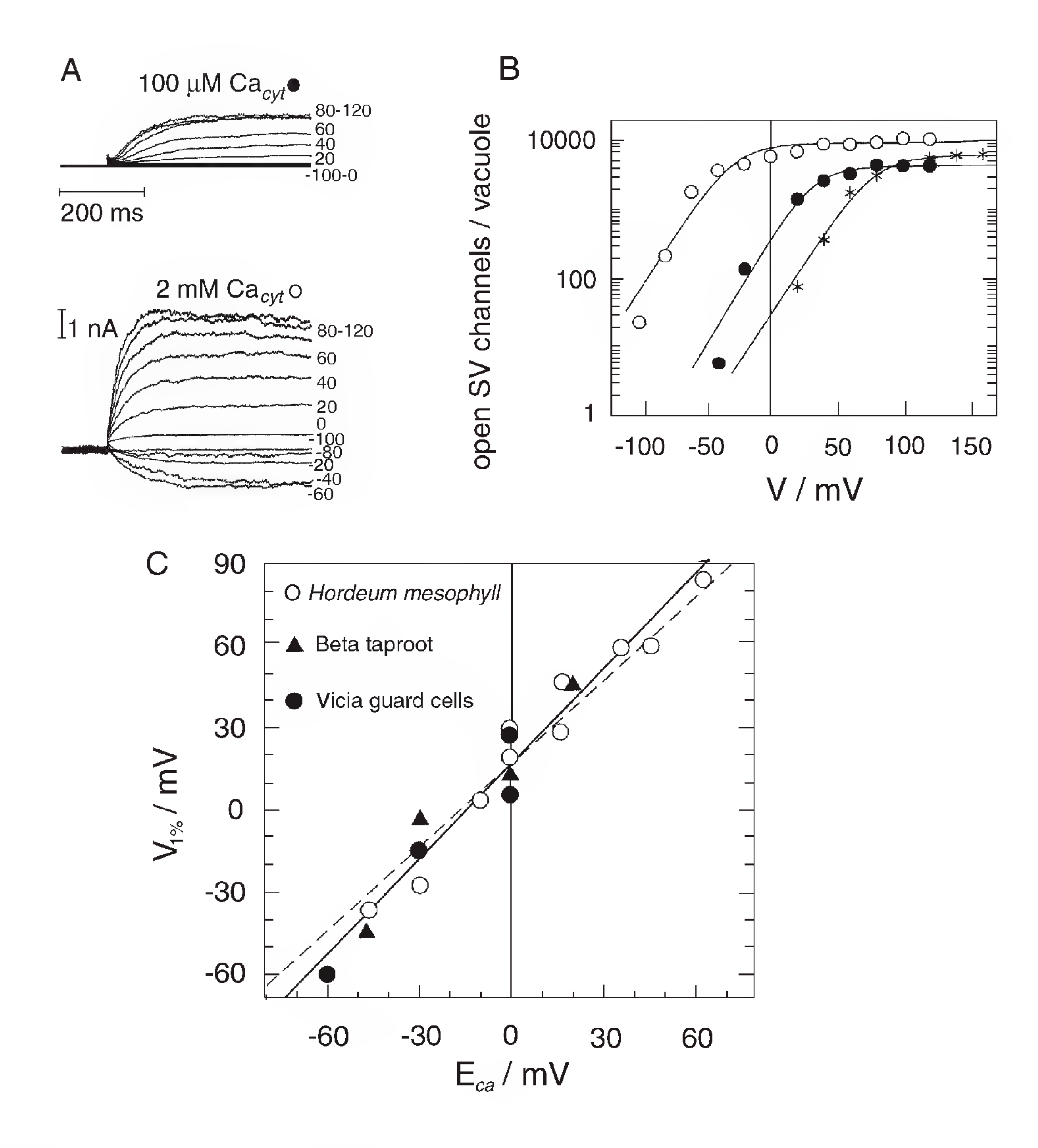


Fig. 5. Modulation of the red beet SV channel by cytosolic and vacuolar calcium. A. Activation of the SV currents in a small (<5 pF) vacuolar vesicle at two different levels of free cytosolic Ca^{2+}. The internal vacuolar solution was 100 mM KCl with nominally zero (~5 nM) free Ca^{2+}. The external solution contained 100 mM KCl and free Ca^{2+} as indicated. From the holding level of –100 mV (cytosol negative), voltage was stepped in 20 mV steps to voltages indicated at the right hand side of each current trace. An increase in cytosolic Ca^{2+} from 100 µM to 2 mM causes a shift of activation threshold to negative potentials and an inward (cytosol-directed) cation current as low as –80 mV may be recorded. B. Voltage dependence of the channel gating was calculated from the data in A and is presented as the mean number of the SV channels per typical vacuole (20 µm in diameter) as a function of transmembrane potential. Hollow circles indicate zero Ca^{2+} in the vacuole and 2 mM Ca^{2+} in the cytosol, filled circles- zero Ca^{2+} in the vacuole and 100 µM Ca^{2+} in the cytosol. For comparison, the data from the experiment in Fig. 3D are replotted (2 mM Ca^{2+} at both membrane sides, asterisks). Solid lines are modified Boltzmann functions fitted to the data. C. Potential activating 1% of the available SV channel's pool is linearly related to the Ca^{2+} equilibrium potential (E_{Ca}=29.5 mV*log $[Ca^{2+}]_{vac}$/ $[Ca^{2+}]_{cyt}$) for three different plant preparations. Potential, activating 1% of SV channels was evaluated by fitting of the voltage dependence at each Ca^{2+} gradient by Boltzmann function. Data for *Hordeum* and *Vicia* are taken from Pottosin et al. (1997), while data for *Beta* from Pottosin et al. (2000b) A dashed line corresponds to the slope factor 1.0.

a slope factor ~1 (Fig. 5C). Thus, at a zero Ca^{2+} concentration gradient, 1% (~ 10^2) of the channels will be open at the potential 17 mV above the E_{Ca}, thus mediating net Ca^{2+} uptake, not a release. Extrapolation of the relation to physiological Ca^{2+} gradient range (10^{-6} M Ca^{2+} in the cytosol, 10^{-3} M Ca^{2+} in the vacuole, membrane potential ~0 mV) resulted in less than 1 open SV channel per vacuole, which apparently leaves insufficient room for its participation in CICR. This conclusion has been confronted by Bewell and co-workers (1999), who have reported substantial $^{45}Ca^{2+}$ release from isolated vacuolar vesicles under the conditions favoring SV channel opening. Unfortunately, due to poor Ca^{2+} buffering, the principal result of this work should be questioned. It appears that Ca^{2+} contamination of the experimental medium could reach several tens of µM. In parallel with the tracer release from pre-loaded vesicles a larger, albeit invisible influx of unlabelled Ca^{2+}, driven by artificially adjusted cytosol-positive diffusion potential, could take place. Hence, in reality it would be a net Ca^{2+} uptake rather than a release. Therefore, the cytosolic Ca^{2+} and voltage may not be efficient in opening of a critical (for the CICR) number of SV channels, and there must be additional "helper" factors at work. Calmodulin could be one of intracellular agents, sensitizing the SV channel to the cytosolic Ca^{2+}, albeit the sensitization effect was moderate, 3-fold at 2.5 µM Ca^{2+} (Bethke & Jones, 1994).

pH. Lowering the cytosolic pH inhibited both SV and FV channels but activated VK channels (Ward & Schroeder, 1994; Schulz-Lessdorf & Hedrich, 1995; Allen et al., 1998a). These changes were moderate, about 2-times for all three channels in the physiological pH range (7.0-7.5). Nevertheless, opposite pH effects on VK and FV channels along with their opposite dependence on cytosolic Ca^{2+} may have an important consequence for the switching of K^+ fluxes between these two channels. It is known that in cold-acclimated plants ABA-induced stomatal closure is not accompanied by a rise in cytosolic Ca^{2+} (Allan et al., 1994). Under such circumstances, vacuolar K^+ release could be dominated by FV channels, activated by cytosol alkalinization (Fig. 2), whereas the highest contribution of VK channels is expected under elevated cytosolic Ca^{2+} conditions (Allen et al., 1998a).

SV channels are inhibited also by vacuolar protons with an apparent pK of 5.0, close to the normal vacuolar pH (Schulz-Lessdorf & Hedrich, 1995). However, due to the competitive effect of vacuolar Ca^{2+}, the effect of pH change from 7.5 to 5.5 is negligible at free vacuolar Ca^{2+} level $\geq$ 50 µM (Pottosin et al., 1997). One may expect that in vacuoles of acidic fruits (pH < 3), regulation by vacuolar protons could have larger impact. In CAM plants diurnal changes of vacuolar pH between 5.5 and 3.3 are observed, with minimal pH correlating to a maximum malate level. For CAM plants grown in a low Ca^{2+} medium, the pH-minimum also is paralleled with the decrease of the free vacuolar Ca^{2+} (Meyer & Popp, 1997). However, in vacuoles of CAM plants the free Ca^{2+} level is always high, from several mM (plants in low Ca^{2+} medium) to 100 mM. Thus, the inhibitory effect of Ca^{2+} would always override that of H^+.

Magnesium. Mg^{2+} content in plant cells, in contrast to total Ca^{2+}, is a function of the ambient concentration, and excess Mg^{2+} is deposited in vacuoles. In barley leaves total Mg^{2+} ranges from 2 mM to 17 mM depending on the cell type (Dietz et al., 1992) which is comparable to 17-20 mM for a typical mammalian cell (Romani & Scarpa, 2000). Free Mg^{2+} in animal cells (0.5-1 mM) is comparable to 0.4 mM free Mg^{2+} measured in mung bean root cells (Yazaki et al., 1988). Compared to Ca^{2+}, Mg^{2+} therefore is poorly chelated:

up to 90% in cytosol (mainly by ATP) and by >50% in the vacuole (mainly by phosphate) and there is no large vacuole to cytosol gradient of free Mg^{2+}. Work on vacuoles revealed that Mg^{2+} effects on the FV are comparable to those of Ca^{2+}, whereas modulation of the SV requires much higher concentrations of vacuolar and cytosolic Mg^{2+} as compared to Ca^{2+}. Cytosolic Mg^{2+} at micromolar concentrations stabilized the closed state of the FV channel in vacuoles of barley mesophyll and *Vicia faba* guard cells (Brüggemann et al., 1999b; Pei et al., 1999). Although cytosolic Ca^{2+} has a similar and even slightly higher potency in the same preparation (Tikhonova et al., 1997), this is due to a much higher free cytosolic concentration that is mainly Mg^{2+}, which controls the FV channel activity. Vacuolar Mg^{2+}, similar to vacuolar Ca^{2+} but with a somewhat higher potency, inhibited the inward current through a FV channel (Table 1). On the contrary, vacuolar Mg^{2+} was 100-fold less effective, compared to vacuolar Ca^{2+}, in the down-regulation of SV channels from barley mesophyll (Pottosin et al., 1997, 2000b). On the other hand, cytosolic Mg^{2+} in the low millimolar range, albeit by itself unable to activate SV currents, promotes SV channel opening at micromolar cytosolic Ca^{2+} in guard cells (Pei et al., 1999). Recent studies on beet vacuoles demonstrated that Mg^{2+} activates the SV channels via a negative shift of the voltage threshold. The magnitude of the shift depended on ionic conditions and Mg^{2+} concentration; within the physiological Mg^{2+} range, as compared to zero Mg^{2+}, the shift of voltage dependence was in the range 20-30 mV (Carpaneto et al., 2001; Pottosin et al., 2001).

The opposite effect of cytosolic Mg^{2+} on the SV and FV channels may be a dynamic factor, affecting the balance of ionic currents across the tonoplast, if the intracellular Mg^{2+} is changed in response to an external stimulus. In the animal physiology the role of Mg^{2+} compared to Ca^{2+} as a signaling ion was denied for a long time due to its high and apparently stable internal concentration (0.5-1 mM), low specificity binding and poor compartmentalization. However, recent findings on stimuli-induced free Mg^{2+} changes may revert this view (Romani & Scarpa, 2000). Less is known about Mg^{2+} distribution, free concentration and dynamics in plant cells, although Mg^{2+} is an important regulator or cofactor of many cellular enzymes. The interest in the role of Mg^{2+} in plant cell signaling is strengthened by the finding that the ABA-insensitive phenotype is linked to alterations of *abi1* and *abi2* genes, these encoding closely related isoforms of PP2C, Mg^{2+}-dependent phosphatase. The *abi1* gene product positively regulates the outward rectifier K_{out} and possibly the anion channel of plasma membrane of guard cells (Luan, 1998); both channels are major mediators of salt release in response to ABA. In contrast to their animal counterparts, Ca^{2+}-permeable channels of the plant plasma membrane show a substantial permeability to Mg^{2+} (White, 2000). In particular, the recently discovered hyperpolarization-activated Ca^{2+}-permeable (Ca^{2+}-influx) channel of guard cell plasma membrane, which was specifically activated by ABA (Hamilton et al., 2000), does not differentiate between Ca^{2+} and Mg^{2+} (Pei et al., 2000). Possible consequences could be summarized as follows: Mg^{2+} entering the cytosol via Ca^{2+}-influx channel would stimulate solute efflux across the plasma membrane via increased activity of PP2C, positively modulating the plasma membrane K_{out} and anion channels and supporting the activating effect of Ca^{2+} on the SV channel. This would in turn promote a vacuolar Ca^{2+} release and the maintenance of high cytosolic Ca^{2+} required for the vacuolar K^+ release through VK channels and Ca^{2+}-dependent activation of VCL.

Polyamines. Diamine putrescine (Put^{2+}), polyamines spermidine (Spd^{3+}), and spermine (Spm^{4+}) are ubiquitous in higher plants. They are present both in the cytosol and in the vacuole at concentrations ranging from a few µM to hundreds of µM (spermine) up to 10 mM for putrescine in K^+ deficient plants (Cohen, 1998). At this range of concentration, polyamines affect both FV and SV channels of plant vacuoles (Brüggemann et al., 1998; Dobrovinskaya et al., 1999 a,b; Table 1). Increased cytosolic polyamine concentration decreased the FV channel open probability with a relative potency Spm^{4+} (Kd~5µM) > Spd^{3+} (Kd~100 µM)>>Put^{2+} (Kd~5,000 µM), both in barley mesophyll and red beet storage vacuoles. Therefore, at their resting concentrations, the activity of FV channels is suppressed more than 2- times by Spm^{4+} and Spd^{3+}. Cytosolic and vacuolar polyamines had no effect on the open probability of the SV channel and act as open channel blockers (Fig. 6)[4], with a relative inhibition potency increasing by about 7-fold with the addition of one amine group $Put^{2+}<Spd^{3+}<Spm^{4+}$ (Dobrovinskaya et al., 1999 a, 1999b). Despite the mechanism of action, the appearance of the polyamine effect on the macroscopic (whole vacuole) SV current was as if these substances affected the voltage gating. The physiological action of the polyamine-induced inward rectification (preferential inhibition of the outward, cytosol-directed current, Fig. 6B) may be to reduce the effect of the feed-forward channel activation by released Ca^{2+} with the respective membrane depolarization. A superposition of voltage dependence of the polyamine block on the opposite voltage dependence of the channel gating (Fig. 5B) could confine the channel opening to a narrow voltage range. The voltage-dependent block by polyamines increased at moderate positive potentials at the side of polyamines application, and relieved at larger potentials, suggesting polyamine permeability of the channel (Fig.6). Previously, polyamines have been shown to be transported through the vacuolar membrane of *Arabidopsis thaliana* by cationic channels, which in whole vacuole configuration mediated macroscopic currents with very similar characteristics (time course, voltage dependence) to those of SV current (Colombo et al., 1992). Therefore, polyamines may not only control the cation flow through the SV channel but, in turn, the redistribution of polyamines between cytosolic and vacuolar pools is likely mediated by SV channels. Effects of polyamines on vacuolar cation channels are different from their effects on plant plasma membrane transporters: inward rectifier K_{in} of *Vicia faba* guard cells and H^+-ATPase in rice coleoptile. The first is inhibited and the second stimulated by high (millimolar) concentrations, with only a weak selectivity among polyamines $Spm^{4+}=Spd^{3+}>Put^{2+}$ (Liu et al., 2000; Reggiani et al., 1992). Thus, the increase of putrescine to a millimolar level seems to have a general effect on FV, SV, K_{in} and plasma membrane H^+-ATPase, whereas high spermine and spermidine levels will in first turn effect vacuolar cation channels, and among them preferentially the FV channel. Enhanced cytosolic Spm^{4+} (100 µM) caused a 3 to 10-fold larger decrease in the SV current as compared to the FV channel, whereas high putrescine level (3 mM) inhibited FV and outward SV currents by an average of only 30% and by 70%, respectively (Brüggemann et al., 1998; Dobrovinskaya et al., 1999a,b). In other words, the diamine to polyamine ratio may efficiently control the relation between the FV and SV currents of the

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[4] Polyamines in a similar fashion, but at somewhat higher concentrations, are known to block other intracellular Ca^{2+} release channels, such as the cardiac ryanodine receptor (RyR) channel (Uehara et al., 1996). Submillimolar polyamine levels inhibit cADPR-induced as well as ryanodine- and caffeine-induced Ca^{2+} release in sea urchine eggs (Chini et al., 1995).

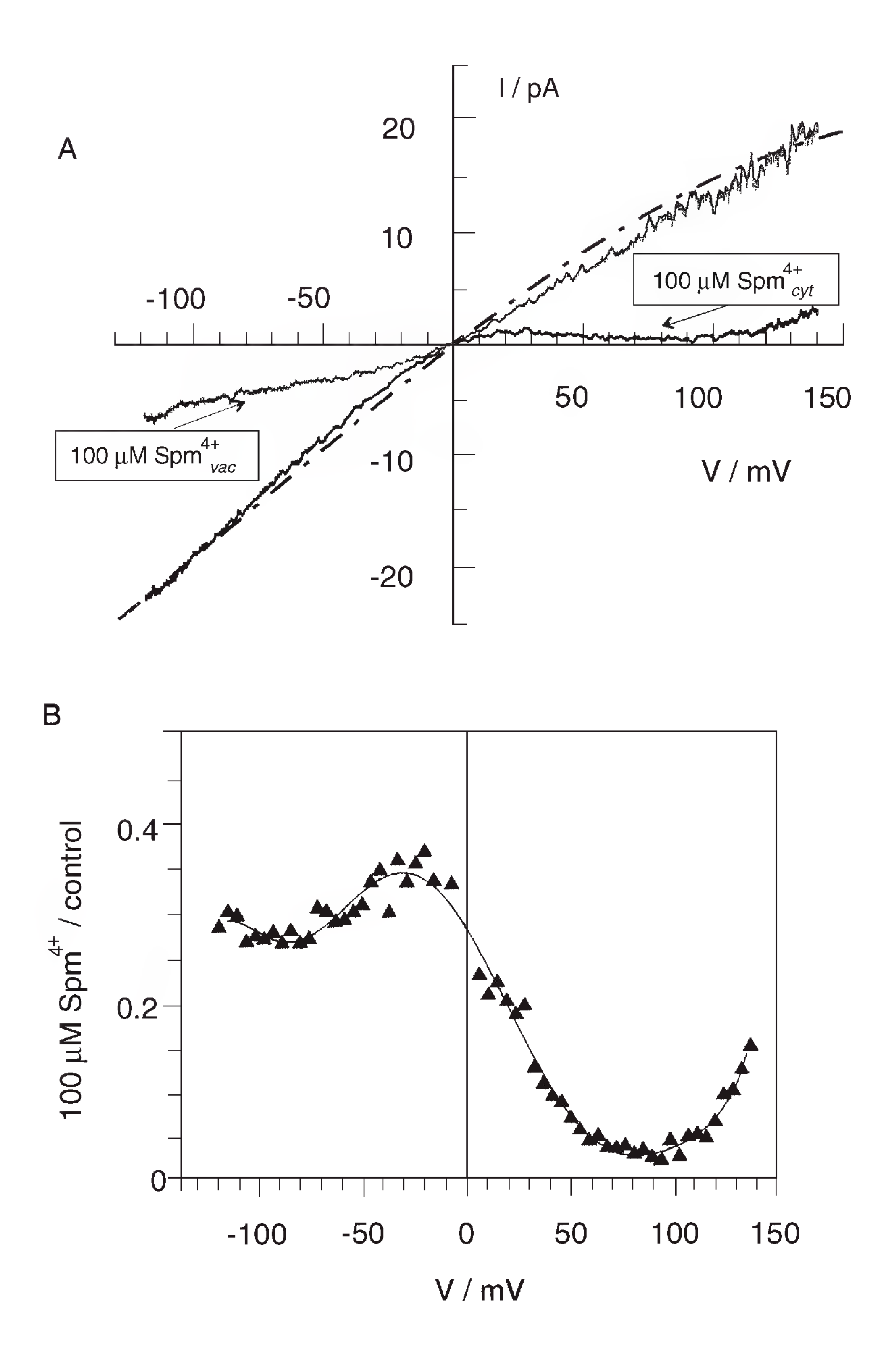


Fig. 6. Cytosolic and vacuolar spermine blocks the red beet SV channel in a voltage-dependent manner. A. Single channel current-voltage relationship in control conditions (symmetrical 100 mM KCl, dashed-dotted line) and in the presence of 100 µM spermine at the cytosolic or at the vacuolar side. A strong voltage dependence of the block is manifested by the fact that cytosolic and vacuolar Spm^{4+} mainly suppress outward or inward currents, respectively. B. Simultaneous effect of the vacuolar and cytosolic spermine on relative current/conductance of the SV channel. The voltage dependence of the effect is dominated by cytosolic Spm^{4+} and may be compared with the opposite gating by membrane voltage (Fig. 5B).

vacuolar membrane. Taking into account the selectivity properties of the vacuolar channels and the ionic gradients, the SV channel may be considered as the Ca^{2+} release channel which, when fully activated, may be also suited for K^+ or Na^+ uptake, whereas the FV channel likely mediates passive exchange of monovalent cations across the tonoplast.

The synthesis of polyamines is known to be induced in plants by various stresses and upon induction of growth and development, but the pattern seems to be stimulus-dependent (Evans & Malmberg, 1989; Galston & Sawney, 1990; Cohen, 1998). A huge accumulation of putrescine (up to 10 mM) without marked changes of higher polyamines levels under K^+ deficiency is an established and universal example (Murty et al., 1971; Crocomo & Basso, 1974; Sarjala, 1996; Watson & Malmberg, 1996). On the contrary, high external K^+ (10-15 mM) depresses putrescine synthesis and stimulates its conversion to higher polyamines (Aurisano et al., 1993; Reggiani et al., 1993). Putrescine feeding under K^+ replete conditions may evoke symptoms similar to K^+ deficiency (Sung et al., 1994). At moderate K^+ starvation, the putrescine accumulation would first switch down the SV channel without marked effect on the FV channel, thus vacuolar and cytosolic K^+ pools would be temporarily in equilibrium (Walker et al., 1996). However, at severe K^+ starvation, an increased putrescine level will also inhibit the FV channel. At these conditions, a driving force for K^+ is directed from cytosol to the vacuole, hence K^+ efflux from the vacuole must be active. On thermodynamic grounds, the active efflux of K^+ can be mediated by a H^+/K^+ symport (Walker et al., 1996) giving rise to cytosolic acidification[5]. This, along with a decreased vacuolar K^+ level (see "Permeable ions"), will further decrease the activity of vacuolar channels.

Putrescine, although at a lower yet millimolar level, is accumulated under anoxia (Reggiani et al., 1992) and osmotic stress (Flores & Galston, 1982; Aziz & Lahrer, 1995). The increase of putrescine titer is common under hypertonic conditions of protoplasts preparation, although it seems to be restricted to monocots (e.g., cereals), whereas various dicots, which are able to regenerate whole plants from leaf protoplasts, respond by a decrease in putrescine and an increase in spermidine and spermine (Tiburcio et al., 1986a,b). Both osmotic and salt stress may cause similar polyamine accumulation, though spermine accumulation, if occurring is characteristic for the salt stress (Erdei et al., 1990,1996). High spermidine and spermine levels in oat (*Avena sativa*) leaves and protoplasts delay osmotic stress-induced senescence (Tiburcio et al., 1994). Resistance to salt stress seems to be correlated with a pattern of polyamine synthesis. For example, salt-tolerant but not salt-sensitive rice (*Oryza sativa*) lines are effective in maintaining a high spermidine and spermine *versus* putrescine ratio (Krishnamurthy & Bhagwat, 1989). Similarly, in seedlings of wheat (*Triticum aestivum*) salt stress causes a slight decrease in putrescine content, an increase of spermidine, and a even larger increase of spermine. However, growth of wheat seedlings was negatively correlated with higher polyamine concentrations (Reggiani et al., 1994). In this case the accumulation of spermine, up to 500 µM in stems of the most salt sensitive wheat line, was obviously already beyond the range of positive regulation. Such a huge concentration will inevitably inhibit not only the

[5] At extreme K^+ deficiency, K^+ uptake across the plasma membrane needs to be maintained by high-affinity K^+ transport, whereas K_{in} channels, due to the outward K^+ gradient, will operate as K^+ leak. Thus, their inhibition by high putrescine (Liu et al., 2000) may be very useful, especially keeping in mind the activation of the K_{in} current by cytosol acidification (Grabov & Blatt, 1997).

FV channel but also the SV channel (Table 1), would therefore reduce or prevent Ca^{2+} release from the vacuole. It is known that salt tolerance is critically dependent on the function of the so-called *SOS* cascade, where one of the key components (protein kinase *SOS2*) is activated in a Ca^{2+}-dependent fashion (Halfter et al., 2000). Ca^{2+} release from the vacuole may not only be involved as an immediate reaction to salt or hypertonic stress (Knight et al., 1997), but also may contribute to the activation of *SOS* pathway at later periods. On the other hand, down-regulation of the FV channel alone by moderate spermine increase (and by Na^+, Fig. 5) during salt stress might help prevent the passive leaking of Na^+ sequestered in the vacuole. At the same time, the SV current in the proximity of its reversal potential would not be significantly affected. Therefore, it might even contribute to Na^+ uptake into the vacuole in exchange for Ca^{2+} release.

Overall, there is a correlation between the level of polyamines and plant development. Generally high spermine and/or spermidine levels are found in young, intensively grown tissues, such as embryos axes or root apices, whereas putrescine is accumulated at later stages; in dying tissues it may amount up to 20% of total nitrogen content (Lin, 1984; Shen & Glaston, 1985; Flores, 1991; Cohen, 1998). Inducible overexpression of arginine decarboxylase in transgenic tobacco plants results in increased putrescine content and phenotypic alterations such as short internodes, thin stems and leaves, leaf chlorosis, and reduced root growth (Masgrau et al., 1997). Plant regeneration from callus culture of rice may be improved by blocking putrescine synthesis and/or applying spermidine treatment, thus causing an increased Spd^{3+}/Put^{2+} ratio (Bajaj & Rajam, 1996). Although the toxic effect of high putrescine levels on vegetative growth and its reversal by restoring a higher polyamine to putrescine ratio is commonly observed, the mechanism of this relationship is not established. The phenotypic alterations in transgenic tobacco due to an overexpression of arginine decarboxylase and a respective increase of putrescine level are very similar to those of vacuolar Ca^{2+}/H^+ antiporter overexpression, when Ca^{2+} becomes unavailable to all but the vacuolar lumen of the cell. (Masgrau et al., 1997; Hirshi, 1999). Because the SV channel is the only firmly established Ca^{2+}-permeable channel of the vacuolar membrane, one might relate putrescine toxicity to SV channel inhibition. This would make the vacuolar Ca^{2+} pool unavailable, thus mimicking symptoms of external Ca^{2+} deficiency. However, different intracellular Ca^{2+} pools may be at work. The apical gradient of a Ca^{2+} increase alone is sufficient to establish and maintain the site of tip growth during the polarized growth of pollen tubes and root hairs. Internal Ca^{2+} stores localized in this region are likely IP_3-sensitive ones of endoplasmic reticulum origin, whereas the vacuole located at the rear part of the cell seems to take no part in localized Ca^{2+} changes (Trewavas & Malhó, 1997; Yang, 1998). The situation is different in meristematic cells, where clusters of provacuoles could come into the contact with endoplasmic reticulum (Marty, 1999). During the induction phase of the growth of transformed of roots *Catharanthus roseus*, an increase in the level of polyamines and of phospholipase C (PLC) activity are positively correlated (de los Santos Briones et al., 1997). Spermine, but not putrescine or spermidine, caused up to a 4-fold stimulation of membrane-bound PLC at concentrations between 10 and 100 µM as compared to a 50 to 160 µM Spm^{4+} increase during the induction phase, whereas higher concentrations of Spm^{4+} produced a strong inhibition (Echevarria Machado & Hernández Sotomayor, personal communication). These initial studies provide a link between increased polyamine levels in intensively growing tissues and Ca^{2+} mobilization from intracellular IP_3-

sensitive stores. In this regard, the investigation of the effects of polyamines, especially of spermine, on the dynamics of intracellular Ca^{2+} and the interaction between different Ca^{2+}-release pathways in plants is of great interest. Particularly, the polyamine stimulation of the PLC and the parallel block of the SV channels may, depending on the localization of IP_3 receptors, initiate a cross-talk via an increase in cytosolic Ca^{2+} between these two Ca^{2+}-permeable channels, or, alternatively, a spatial redistribution of the Ca^{2+} signal to the vicinity of IP_3-sensitive stores.

CONCLUSIONS

Four distinct ion channels SV, FV, VK and VCL/ VMal, two types of H^+ pumps, and several metal/H^+ antiporters have been characterized in the tonoplast of higher plants (Fig. 7). Thus, the basis for the generation of gradients for H^+ and Ca^{2+}, and mechanisms of uptake of polyvalent cations (including heavy metals) and sodium into the vacuole are understood. Potassium and anions can be actively accumulated into the vacuole, but corresponding transport mechanisms are still elusive. At replete conditions, concentration gradients for K^+ and anions combined with the membrane potential difference favor passive release of these ions from the vacuole to the cytosol (Fig. 1). However, this process is handicapped, owing the voltage dependence of the major ion currents (Fig. 7). The SV and FV channels are outward rectifiers, i.e., are able to mediate mainly cation uptake into the vacuole. This is due to intrinsic gating and inhibition of inward (cytosol-directed) currents by divalent cations from the vacuolar side. Nevertheless, a residual inward current through FV channels is comparable by absolute magnitude to that generated by H^+ pumps. Hence, H^+ pumping into the vacuole could be electrically balanced. The VCL channel favors uptake of anions into the vacuole, whereas outward currents carried by this channel are tiny. Large ion release by vacuolar channels can only take place when the activation of both cation and anion channels shifts towards the narrow voltage region roughly fitting the physiological one, where the condition of net salt (K^+ and anions) release is met (Fig. 7). Such a transition, allowing a massive volume reduction, likely occurs in motor cells, such as guard cells, but the specific mechanisms of up-regulating vacuolar channel activity in the physiological voltage range are unknown. Cation channels are efficiently down-regulated by polyamines, whose concentration tends to increase upon various stresses and growth induction. The cation (SV and FV) and anion channels are gated by permeable ions in a valve-like fashion, which might underlie their role in cell volume regulation.

PERSPECTIVES

Membrane voltage is an important factor which links the activity of membrane transporters, pumps, and channels, as well as defines the direction and magnitude of the vacuolar ion transport. Membrane potential values reported by microelectrode studies did not fit well into the very negative values expected on the ground of energetic considerations for currents generated by H^+ pumps nor did they fit well into the range of potentials delimited by known ion channels. Instead, they lie in between. The compromise

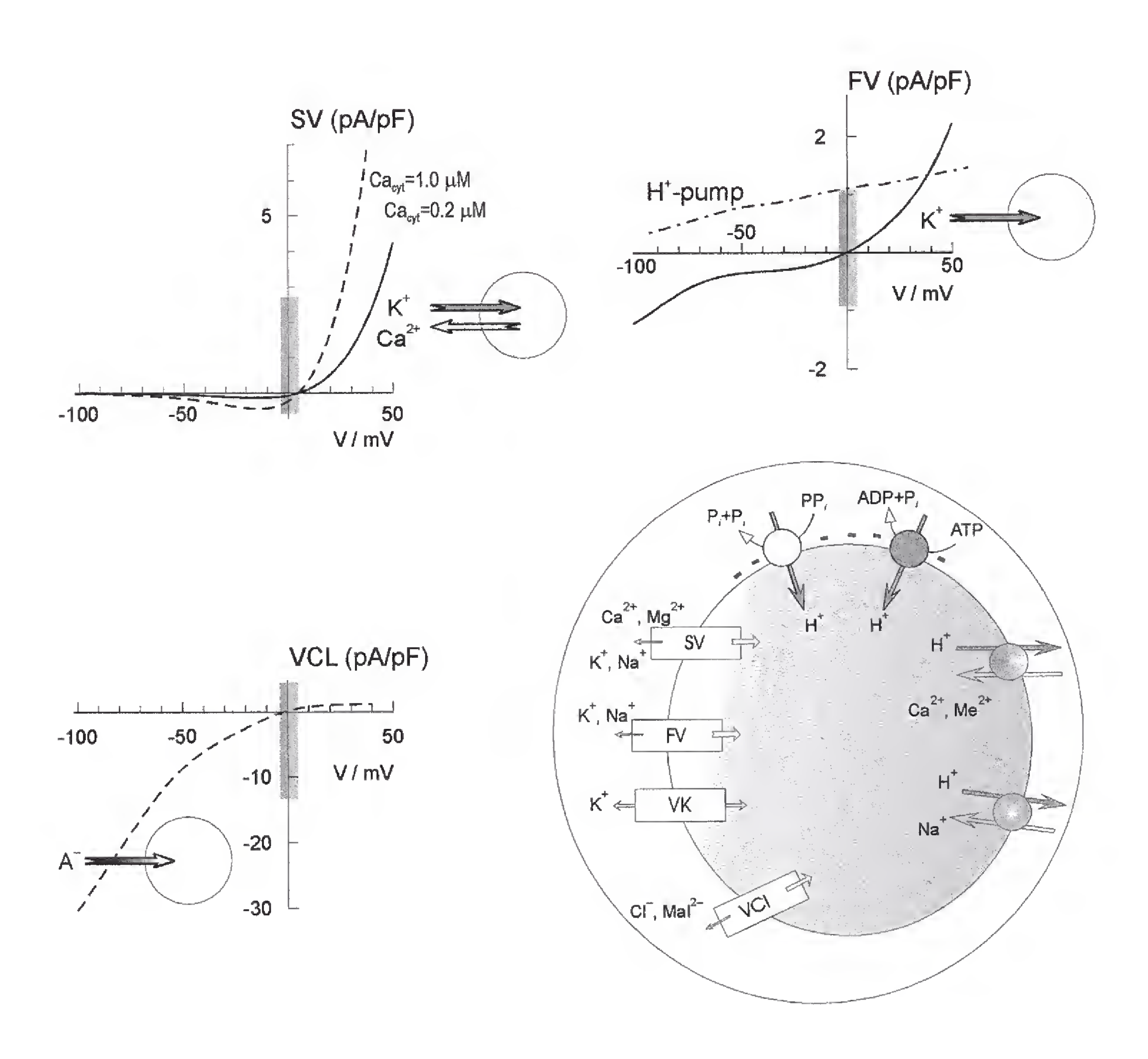


Fig. 7. Summary of the vacuolar ion transporters. Ion channels (SV, FV, VK and VCL/VMal) have been characterized only functionally. H^+-translocating pumps (V-type ATPase and inorganic pyrophosphatase) and several metal/H^+ antiporters (including CAX family members CAX1 and CAX2 transporting Ca^{2+}, Mn^{2+} and Ca^{2+}, closely related AtMHX , transporting Mg^{2+} and Zn^{2+}, and Na^+/H^+ antiporters) have been characterized by means of molecular genetics and by functional assays. Steady-state I/V relations for the SV, FV and anion currents were constructed, assuming 0.5 mM Ca^{2+} in the vacuole and 0.5 mM Mg^{2+} at both sides. The shaded bar indicates the region of net salt (K^+ plus anion) release. See text (Conclusions) for comments.

between active and passive conductance of the vacuolar membrane might be a subject of variation, but the range of possible potential displacements and their dynamics are, in fact, unknown. Are there, for instance, changes in the membrane potential of guard cell vacuoles during stomatal movements? Spectroscopic measurements with potential-sensitive dyes might serve as a useful complement to conventional microelectrode techniques for resolution of these issues.

All plant cells are not alike. There is, for instance, a remarkable difference in solute accumulation between symplastically connected cells of different types within a leaf. Such a difference could only be related to different transport capabilities of respective vacuoles (Karley et al., 2000). Does this mean that there is differential expression or that there are different ways of regulation of tonoplast transporters? In a single plant cell several types of vacuoles are found. Electrophysiologists mainly have studied large central vacuoles. Even though there are at least two distinct types of central vacuoles: acidic, lytic vacuoles containing vegetative-specific γ-TIP aquaporin, and storage vacuoles containing a- or δ-TIPs, respectively (Marty, 1999). Besides, depending on the developmental phase a plant cell can contain a variety of vacuolar “generations”, including tiny provacuoles. Small vacuoles likely form a distinct Ca^{2+} store (Harper, 2001). The vacuole as a dynamic compartment is a subject of biogenesis, differentiation, and remodeling, both morphologically and biochemically. Just how these changes are related to changes in tonoplast transporter repertoires is unknown. Systematic study of vacuolar ion transport in relation to the biogenesis and specific function of vacuoles is awaited.

There are few direct measurements on free Ca^{2+} content in plant vacuoles, and almost nothing is known about free Mg^{2+} in plant cells. Given the strong impact of these ions on the function of vacuolar channels, there is a clear need to fill this gap. Free Ca^{2+} in vacuoles is believed to be in the millimolar range. However, there are indications for vacuoles of barley aleuron tissue free Ca^{2+} might be much lower, down to the nanomolar range (R. Jones, personal communication). Based on the voltage dependence of ion channels in vacuole-attached patches from barley mesophyll, the vacuolar Ca^{2+} level is in the low micromolar range in young plants (Tikhonova, Pottosin & Schönknecht, unpublished result). *In vivo* monitoring of the vacuolar free Ca^{2+} and of its impact on the vacuolar ion transport may open a new perspective for the understanding of ionic homeostasis in the vacuolate plant cell. The vacuolar SV and FV channels are strongly affected by physiological concentrations of polyamines, and therefore they may be prime targets for polyamines in plant cells. Thus, the uneven distribution of polyamines in plant tissues and specific changes of their levels during different stresses and developmental signaling will definitely have an important impact on the vacuolar ion transport and Ca^{2+} mobilization. A causal relationship between polyamine metabolism, Ca^{2+} and ionic homeostasis, and their integration for long term physiological responses could be therefore elucidated.

There has been a great variety of ion channel currents reported for the vacuolar membrane. Apparently, the actual number of *bona fide* vacuolar channels is smaller. In some cases the difference in current characteristics simply reflects different experimental conditions, and there is perhaps a number of sheer artificial currents, not convincingly supported at the whole vacuole nor at the single channel levels. More experimental evidence and stricter criteria need to be applied to resolve this issue. Nevertheless, some ion channel types await their discovery. Particularly, the search of the ion channel

responsible for anion release from the vacuole should be continued, and the presence of tonoplast ligand (IP_3, cADPR)-gated channels needs to be unequivocally demonstrated. As demonstrated by the analysis of the *Arabidopsis* genome database, there are apparently no homologues of animal Ca^{2+} and Ca^{2+}-permeable channels, except those belonging to cyclic nucleotide-gated and glutamate receptor channels' families (Mäser et al., 2001). How this could be reconciled with a variety of Ca^{2+}-permeable channels already functionally detected in membranes delineating different compartments of the plant cell? The molecular identities of vacuolar ion channels, even those best characterized by electrophysiological techniques, the FV and the SV channels, are unknown. This abscence of knowledge, handicaps the understanding of their physiological function. However, a recent study by Schönknecht and co-workers (2002) reported the first vacuolar cation channel homologue and provides an initial clue for its relation to known vacuolar currents.

ACKNOWLEDGMENTS

The present work was supported by the Alexander von Humboldt Foundation, CONACyT (grants 3735PN and 29473N), and Fideicomiso Ramón Álvarez Buylla de Aldana. We are grateful to many colleagues and collaborators for invaluable experimental contributions and stimulatory discussions. Especially, we wish to thank Luba Brüggemann, Oxana Dobrovinskaya, and Gerald Schönknecht, co-authors of joint publications and contributors to unpublished work first presented in this paper; Ileana Machado and Teresa Hernández for communicating unpublished data; the two anonymous reviewers and Humberto Cruzblanca for the critical reading of the manuscript and extensive comments; Nick Varley and Victor Steinmann for their generous help in editing the text.

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Recibido en mayo de 2001.
Aceptado en septiembre de 2002.

Acta Botanica Mexicana (2002), 60: 79-84

Reseña de Libro

Héctor M. Hernández, Alfonso N. García Aldrete, Fernando Álvarez y Miguel Ulloa (compiladores). 2001. Enfoques contemporáneos para el estudio de la biodiversidad. Ediciones Científicas Universitarias. Serie Texto Científico Universitario. Instituto de Biología, UNAM y Fondo de Cultura Económica. México, D.F. 413 pp.

Derivado de la celebración de un simposio para conmemorar el 70 aniversario de su fundación, el Instituto de Biología, de la Universidad Nacional Autónoma de México, publicó la obra *Enfoques contemporáneos para el estudio de la biodiversidad*, compilado por Héctor M. Hernández, Alfonso N. García Aldrete, Fernando Álvarez y Miguel Ulloa. El libro consta de 15 capítulos escritos por investigadores que laboran en el Instituto de Biología o en instituciones del extranjero, en donde se explora el papel que desarrolla la investigación sistemática sobre la conservación de la diversidad biológica. La resolución de esta compleja tarea se aborda a través de una revisión sobre los métodos y fundamentos teóricos de este heterogéneo campo de investigación, considerando los distintos niveles de organización biológica (desde genes hasta ecosistemas), así como distintos grupos de organismos. Sin embargo, si se toma en cuenta la dificultad que existe en la definición del concepto de biodiversidad y la enorme gama de temas que pueden ser desarrollados al respecto, un asunto que llama la atención es la carencia de una presentación que permita entender explícitamente sus propósitos y las razones por las cuales se incluye cada capítulo. Sin este imprescindible marco de referencia, el lector debe de ir de un tema a otro sin nada que permita darle una coherencia temática específica a la obra.

El primer capítulo de este libro fue elaborado por Kevin C. Nixon y Helga Ochoterena, quienes discuten los avances más recientes en los principios metodológicos en la taxonomía cladística, usando el método de la parsimonia para la construcción de hipótesis filogenéticas. Aunque existe una enorme controversia en varios de los puntos que se desarrollan en el texto (p. ej. el uso en estudios cladísticos de atributos moleculares *vs*. morfológicos o la inclusión de la mayor cantidad posible de estados de carácter para realizar los análisis), los autores enfatizan el grado de validez e importancia de la obtención de una determinada hipótesis de filogenia, que posteriormente es usada para decisiones nomenclaturales y de clasificación de un determinado grupo. Esta hipótesis debe corroborarse o refutarse usando otras fuentes de evidencia, particularmente aquellas obtenidas por medio del trabajo taxonómico “tradicional”, el cual se basa principalmente en caracteres morfológicos. Los autores destacan claramente que estos enfoques aparentemente antagónicos son complementarios y esenciales para entender de manera más precisa la filogenia de un determinado taxon.

La segunda contribución fue escrita por Olle Pellmyr, Manuel Bálcazar-Lara y James Leebens-Mack, en la que se desarrolla la importancia de las interacciones de especies y la coevolución en la generación de la biodiversidad, documentando si en la relación mutualista establecida entre las plantas del género *Yucca* y sus insectos polinizadores (especies de los géneros *Tegeticula* y *Parategeticula*), la evolución de caracteres asociados a la dieta de los insectos es causada por la selección que impone

la morfología floral y no por factores extrínsecos a la interacción. Los autores destacan que para analizar el papel histórico de la coevolución en la generación de la biodiversidad en asociaciones mutualistas se requiere de filogenias robustas, además de que es necesario reconocer los fenómenos de coevolución verdadera y conespeciación (cladogénesis paralela).

David M. Spooner y Sabina Lara-Cabrera escribieron la tercera contribución en la que se enumeran los aportes que distintos enfoques de la sistemática molecular han generado para una mejor comprensión de la compleja historia evolutiva de las plantas cultivadas, a través de una valiosa revisión bibliográfica sobre el tema (1992-1999). Esta información es resumida en un cuadro donde se detalla la información de las distintas técnicas moleculares aplicadas por cultivar, indicando la(s) cita(s) correspondiente(s). El capítulo finaliza relatando la contribución específica de estos trabajos agrupándolos con base en su principal objetivo de investigación, por ejemplo, aquellos que encaran preguntas relacionadas con la sistemática o con el mejoramiento de bancos genéticos.

El tema sobre la contribución de los caracteres anatómicos en la diversificación de la familia Cactaceae es desarrollado por James D. Mauseth, a través de una detallada discusión entre los atributos anatómicos de ciertos órganos y la función que desempeñan, poniendo especial atención al polimorfismo del tejido leñoso y al origen de las estructuras reproductoras. El principal interés del autor es destacar que a diferencia de los estudios moleculares que están actualmente en boga, el enfoque anatómico ha despertado habitualmente poco interés entre los nuevos taxónomos, sin embargo, esta disciplina tiene protocolos muy sencillos y de bajo costo económico, que también pueden aportar información valiosa para el entendimiento de los aspectos ecológicos, fisiológicos y evolutivos de las cactáceas en particular y de las plantas en general.

El quinto capítulo del libro fue escrito por Kent E. Holsinger, quien describe la relación entre la diversidad de las angiospermas y los sistemas de apareamiento y de biología de la polinización. El autor realza la variedad de mecanismos a través de los cuales las plantas con flor se reproducen por fertilización cruzada, minimizando los eventos de autofertilización. El análisis de las ventajas y desventajas evolutivas de ambos procesos constituye una buena parte del contenido de esta contribución y la revisión de literatura es amplia. Holsinger propone que para avanzar en el entendimiento de estos complejos sistemas de apareamiento es necesario resumir y unificar las aportaciones de la genética molecular de poblaciones, genética cuantitativa y ecología de poblaciones.

Por su parte, Gustavo Hormiga y Jonathan A. Coddington documentan los patrones filogenéticos relacionados con la diferencia de tamaño que presentan las hembras y los machos de las arañas tejedoras de telas orbiculares (Araneae, Orbiculariae), analizando este carácter en 536 especies. En general el dimorfismo sexual de tamaño es más común en organismos marinos y las arañas son uno de los escasos ejemplos de animales terrestres que presentan este atributo, existiendo especies donde la relación entre el largo del cuerpo de las hembras con respecto al de los machos es de hasta 12:1. Mediante un análisis cladístico de los géneros, los autores demuestran que el dimorfismo sexual en el grupo obedece a un fenómeno de gigantismo de la hembra más que a un enanismo de los machos, definiendo nueve posibles trayectorias evolutivas, sin que exista un modelo generalizado que permita explicar su origen y mantenimiento en términos ecológicos y evolutivos, por lo que sugieren su análisis individual desde una perspectiva histórica.

La diversidad de anfibios en México es la más alta del planeta y es por ello que resulta particularmente interesante la contribución que desarrollan David M. Green, Robert L. Carroll y Víctor-Hugo Reynoso sobre los patrones de extinción en anfibios considerando sus tendencias pasadas y actuales. En este capítulo se fundamenta que actualmente las poblaciones de anfibios están declinando debido principalmente a la destrucción o alteración de su hábitat. Los autores destacan la importancia de evaluar atributos demográficos de las poblaciones de anfibios para predecir su declinación, particularmente la determinación de sus tamaños efectivos. Lo anterior debe de acoplarse con acciones de conservación de los hábitats y la implementación de corredores entre las reservas, particularmente en ambientes de montaña y en comunidades tropicales, los cuales presentan un gran número de taxa endémicos. Sin duda alguna, lo expresado anteriormente aporta argumentos que deberían de ser considerados en las políticas de conservación de este importante grupo faunístico.

El octavo capítulo de este libro fue escrito por Wayne Maddison y Tila M. Pérez, quienes describen la importancia del estudio de la evolución de un carácter en un linaje específico para determinar sus relaciones de parentesco. Se documenta este tipo de fenómenos en el comportamiento del cortejo en arañas saltadoras y en ácaros plumícolas, combinando el análisis de la historia de los caracteres y los eventos que se observan en la naturaleza. El ensayo termina con una reflexión acerca de la necesidad de seguir inventariando los seres vivos del planeta.

Marjorie L. Reaka-Kudla desarrolla un interesante ensayo sobre la biodiversidad y la conservación de los arrecifes de coral, uno de los ecosistemas más diversos de la Tierra, el cual está insuficientemente documentado. Un ejemplo muy claro sobre el desconocimiento de los ambientes marinos es que entre 1983 y 1997 fueron descubiertos tres phyla de invertebrados marinos: Loricifera, Vestimentifera y Cicliophora. Reaka-Kudla intenta un cálculo sobre la diversidad de los arrecifes coralinos con base en la relación especies/área y otros supuestos teóricos, arribando a una cifra entre uno a cinco millones de especies descritas y no descritas; si estas cifras son correctas, el número de especies descritas actualmente para los arrecifes coralinos sería en el mejor de los casos cercano a 10%. La cifra anterior es extremadamente preocupante si se considera que solamente 30% del área de este ecosistema puede considerarse sin amenazas para su conservación apropiada. La autora finaliza con una reflexión sobre un cambio en las políticas de manejo y conservación de tales ecosistemas, modificando la perspectiva local o regional a una escala mundial, donde las áreas prioritarias podrían ser elegidas con base en los métodos sugeridos por el análisis de discontinuidades (gap analysis).

Desde otra esfera de estudio, Daniel R. Brooks, Virginia León-Regagnon y Gerardo Pérez-Ponce de León resaltan el valor que los parásitos tienen para lograr un manejo sustentable de la biodiversidad, a través de la información que éstos brindan sobre la historia natural del hospedero y de las relaciones tróficas del ecosistema en que se encuentran. El trabajo destaca además la enorme importancia que tienen los taxónomos en la cuantificación más adecuada de la biodiversidad planetaria, discutiendo en particular su papel en los inventarios biológicos tradicionales de los parásitos, los cuales pueden estar asociados a estudios biogeográficos, ecológicos y evolutivos. Los autores sugieren que si se incrementan las actividades de inventario de la biodiversidad, de elaboración de clasificaciones predictivas y de manejo electrónico de la información

taxonómica a través de bases de datos, puede revertirse el inadecuado manejo de la biodiversidad que el ser humano ha hecho hasta el momento.

Una contribución respecto al grado de confiabilidad de los supuestos en los que se apoyan las estimaciones sobre la diversidad mundial de los hongos es presentada por D. Jean Lodge. Un primer supuesto indica que entre los hongos la especificidad o preferencia del hospedero puede ser distinta entre especies de comunidades templadas y tropicales, un patrón que no ha sido encontrado en los estudios revisados por el autor para hongos fitopatógenos, endófitos y desintegradores de la hojarasca. Otro supuesto adjudica similares patrones de distribución geográfica entre los hongos y las plantas superiores, lo cual no ha sido corroborado para los trópicos. Para evaluar lo anterior, Lodge analiza la distribución de 276 especies y variedades de la familia Xylariaceae, que es un grupo relativamente bien representado en las colecciones de hongos tropicales. Lamentablemente, Lodge ubica estos taxa en una tabla con categorías confusas, que no se distinguen claramente entre sí (p. ej. Nuevo Mundo, Neotropicales y Sudamericanas), concluyendo que el tipo de distribución geográfica más común en este grupo es el regional, siendo resultado de procesos biogeográficos de tipo histórico y ecológico. Esta parte resulta especialmente confusa y sus aportaciones sobre este interesante tema son entonces limitadas.

María de los Ángeles Herrera-Campos y Thomas H. Nash III abordan de manera detallada aspectos sistemáticos, de diversidad y de filogenia de una de las formas terrestres menos conocidas pero, paradójicamente, de las más numerosas, heterogéneas y ampliamente distribuidas de nuestro planeta: los líquenes. Los autores aportan una sección donde se describe pormenorizadamente la diversidad morfológica del talo de los líquenes en cuanto a color, forma, tamaño y sustrato que ocupan, indicando que se estima que existen entre 13,250 y 17,000 especies, una cifra que debe de ser manejada de manera cuidadosa, ya que existen muchos países donde el grupo se ha colectado poco, además de que los criterios diagnósticos para la delimitación de las especies han sido tradicionalmente morfológicos y sólo recientemente han empezado a incorporarse datos biogeográficos o moleculares. La contribución finaliza con la inestimable información sobre la clasificación genérica del grupo para México, basada en trabajos florísticos aislados, que demuestran la necesidad de seguir desarrollando estudios que documenten de mejor manera la riqueza liquénica del país.

La relevancia actual y futura de los proyectos florísticos en la sistemática y la conservación de la biodiversidad es presentada por Sandra Knapp, Gerrit Davidse y Mario Sousa Sánchez, destacando básicamente sus aportaciones a tres niveles: 1) el incremento de los acervos de colecciones científicas para determinar la diversidad morfológica de las especies y precisar sus áreas de distribución geográfica para establecer sus prioridades de conservación, 2) la capacitación de nuevos botánicos, pero especialmente taxónomos y 3) la difusión de la información sistemática, comprensible para la sociedad, pero particularmente para los tomadores de decisiones en el manejo de la biodiversidad, para lo cual resultan especialmente útiles los sistemas electrónicos (p. ej. internet). Como ejemplo de lo anterior se muestra la naturaleza y los alcances del proyecto Flora Mesoamericana. Un punto que llama la atención es que a pesar de lo mencionado por los autores en el punto dos, durante el desarrollo de este proyecto resulta palpable la escasa participación de estudiantes de posgrado mexicanos (con sólo una tesis de doctorado y tres tesis de maestría), un problema muy grave que debería de ser

resuelto en el menor plazo posible. También resulta paradójico que los autores enfaticen tanto la importancia de la transmisión de la información de la flora por internet y que la dirección de la página electrónica que citan para examinar los avances de esta flora no sea correcta (www. fm) y su consulta nos lleve a conocer los atractivos turísticos de una isla del Pacífico (Micronesia).

Por otro lado, Víctor Sánchez-Cordero, A. Townsend Peterson y Patricia Escalante-Pliego describen su interés por la elaboración de modelos para determinar la distribución potencial de especies y su aplicación en la conservación de la diversidad biológica. Los autores sugieren que la orientación de la investigación para encarar la pérdida de biodiversidad mundial debería enfocarse a conocer su magnitud, en dónde se distribuye y cómo conservarla adecuadamente. Se hace una reflexión sobre el papel fundamental que tienen al respecto las colecciones científicas y las actividades de inventarios biológicos, discutiendo los sesgos de su información y lo incompleto de los inventarios de biodiversidad, aun para grupos relativamente bien conocidos taxonómicamente como aves y mamíferos. Para revertir esta situación sugieren incrementar los inventarios biológicos y elaborar modelos de distribución de las especies con base en la información aportada por los especímenes depositados en las colecciones científicas, indicando para estos últimos sus ventajas y desventajas, recomendando particularmente los modelos de algoritmos genéticos.

La última contribución del libro pertenece a Paul H. Ehrlich, quien discute el papel de la sistemática en la conservación de la biodiversidad, mediante el uso de grupos indicadores, sin señalar de manera exacta cómo deben de ser elegidos estos grupos ni la forma en que pueda probarse su representatividad para definir tendencias de riqueza regionales. Ehrlich critica la actividad de los taxónomos cladistas, ya que los resultados que ofrecen con respecto a su costo son bastante limitados para ofrecer alternativas útiles en la conservación de la diversidad biológica. La crítica se extiende a los taxónomos en general, ya que considera que no han sido capaces de aportar sistemas de clasificación más estables. Esta aseveración parece demasiado severa si se considera la gran complejidad que representa el estudio de la clasificación del mundo viviente y el escaso apoyo económico que tradicionalmente se le ha asignado. Por último, el autor coincide con otras opiniones contenidas en capítulos previos del libro acerca de la necesidad de mejorar la transmisión del conocimiento sistemático e incrementar la formación de recursos humanos en taxonomía.

Es lamentable que al final de la obra no exista una contribución por parte de los compiladores que destaque y resuma las aportaciones que el libro integra sobre el complejo mundo del estudio de la biodiversidad y los retos de investigación que plantea al respecto, tanto en una perspectiva nacional como internacional. Por ejemplo, es claro que distintos autores coinciden en la importancia que los taxónomos, las colecciones científicas y los inventarios biológicos tienen para la generación de alternativas para encarar la crisis por la que actualmente atraviesa la biodiversidad. Esta carencia podría haberse minimizado si hubiera existido una lectura de los capítulos entre los autores del libro. Confío en que las coincidencias que detecto a través de la lectura del libro sean reales y que los autores, dentro de sus instituciones respectivas, lleven este ejercicio de reflexión a la práctica, promoviendo el desarrollo de los diversos campos de la sistemática de la fauna y de la flora de México y del mundo. Finalmente, espero que este libro se distribuya adecuadamente, ya que su gran diversidad temática y las excelentes revisiones

bibliográficas que caracterizan la mayoría de sus capítulos lo convierten en una obra de consulta obligada para distintos cursos de licenciatura y posgrado de México. Considero que esta obra será también útil a personas interesadas en adquirir marcos teóricos para entender la complejidad existente en el diseño de estrategias de manejo de la biodiversidad, que desde su concepción más amplia incluye el aprovechamiento, rehabilitación, restauración y conservación de los recursos naturales.

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México

Suscripción anual:

México $ 100.00
Extranjero $ 30.00 U.S.D.